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Growth, 1941, **5**, 119-121.

THE EFFECT OF DAILY ADMINISTRATION OF INSULIN ON GROWTH OF THE WHITE RAT

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(Received for publication, February 3, 1941.)

Insulin therapy has become more important and of more interest to a greater number of people in recent years than ever before. This has come about through the use of insulin for various conditions other than that of diabetes mellitus. It is now being recommended and rather widely used in mental therapy and in nutrition to increase the appetite of certain types of underweight persons. What the effect of "sub-shock" doses over long periods of time will be has never been well determined. The work reported here was an endeavor to study the effect of daily injections of insulin[2] (Iletin, Lilly) on the growth of the white rat.

A survey of the literature reveals a wealth of material regarding the action of insulin on various reactions and functions of the body, but very little on growth as such. Various workers (Fleiderbaum, 1933; Bramwell, 1904; Allen, 1922) have reported isolated cases of insulin or pancreatic infantilism, but these were of a clinical order, few in number, and not controlled.

A. PROCEDURE

Rats of known ages were obtained from the Wistar Institute stock to begin the colony. First litters born to these animals were taken to represent the first generation. At birth all litters were cut to six in number. These were weaned at the age of 24 days and insulin injections (intraperitoneally) begun. From a trial and error method, a dosage of 20 units per kilogram of body weight per day was selected for the majority of the experimental animals. Such amounts did cause some animals to go into shock and a few of them were lost from time to time. Another series was given 40 units per kilogram of body weight per day. This was given in two injections daily (except Sun-

[1]The author wishes to express his appreciation to Dr. H. D. Bruner of the University of North Carolina for his valuable aid in the statistical computations involved.
[2]Acknowledgment is made of the generous supplies of insulin furnished by Eli Lilly and Company.

day), one in the morning and the other in late afternoon. Food (Purina Chow) and water were available at all times and green vegetables were fed twice per week.

The injections were given daily, except Sunday, from the age of 24 days until after the animals had reproduced and the litters weaned. Animals were allowed to breed as early as possible, males and females being kept in each cage until the females were known to be pregnant. Life histories were kept on each animal. Some of the control animals were injected daily, except Sunday, with 0.9 per cent salt solution in amounts comparable to the insulin given the experimental animals, while others received no injections.

Five successive generations comprising 206 animals were injected with insulin. Control animals numbering 292 were studied concurrently. The work was begun in October, 1937, and completed in October, 1940.

The weight of each animal, control and experimental, from birth until sacrificed was recorded every third day. The data so obtained were subjected to both graphic and statistical analysis. *Throughout the analyses the sexes were treated separately.* The arithmetical means and standard deviations of the weights at the same ages were computed for each generation on both control and experimental groups. Within the same generation, the data on the experimental animals were compared with those on the control animals by use of the standard error of the differences of two means. Finally, the data on generations three and five were compared with those of generation one by the standard error of the means.

B. Results

Comparison of the graphed data suggested that there were no significant differences between the injected and control groups. However, in view of the fairly high degree of scatter in both groups, a statistical analysis was desirable.

Of the 26,220 weights collected into 532 age groups only nine weights scattered through the five generations were outside the limits of the mean $\pm$ three standard deviations and of these nine, seven were control females.

Use of the standard error of the differences of two means shows that the difference between the means of the data on the injected animals and of the data on the control animals (same age, sex and generation) could arise by chance variation (99.7 chances in 100) in all but 30 of

266 comparisons. Of these 30 exceptions, 9 were in the control group and 21 were in the experimental group. Using the same method to compare Generation 1 with Generation 3 and Generation 1 with Generation 5, it was found that the observed differences could occur by chance except within the female experimental group. In this group, the injected females of Generation 1 were significantly heavier than those of Generations 3 and 5 at respective ages. The significance of these differences in mean weight lies in the later onset of reproductive activity of Generations 2 through 5. This point will be the subject of a separate report. Furthermore, with the exception noted, the trends of the graphed data were not constant irrespective of the lack of significance of statistical analyses.

It is noteworthy that the birth weights of the young born of the fourth generation of insulin injected mothers were not significantly different from those of either intra- or inter-generation newborn.

C. Conclusion

The amounts of insulin used are definitely greater in proportion to body weight than have been used over long periods of time therapeutically. From this study it can only be concluded that insulin given in amounts of from 20 to 40 units per kilogram of body weight per day has no effect upon the weight increment (as a criterion of growth) of the white rat, either in one generation or in succeeding generations through five.

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Growth, 1941, **5**, 123-139.

STIMULATION OF THE PROLIFERATION OF NEURONS BY THE GROWTH HORMONE: I. EXPERIMENTS ON TADPOLES[1]

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(Received for publication, February 4, 1941.)

A. INTRODUCTION

In the previous paper (Zamenhof, 1940) the author investigated the present possibilities of effecting such artificial changes in the architectonic of the (human) cortex as would result in an increase in higher brain functions. It was demonstrated that the number of neurons, especially in the asensoric and amotoric areas is, next to the complexity of nerve fibres, one of the most important factors in determining the level of higher brain functions. In contradistinction to the complexity of nerve fibres which cannot be influenced artificially with the existing knowledge, the number of neurons can be probably artificially increased. This may be possible by stimulating the proliferation of prospective nerve cells in the early period when they have not yet lost their ability to divide. As demonstrated in the preceding paper, and as it will be demonstrated later in this paper, the so-called growth hormone preparation of the eosinophile (acidophile) cells of the anterior lobe of hypophysics seems to be able to stimulate the proliferation of cells in this early period.

The object of this investigation, first in the projected series, is to prove experimentally that part of the above theories which relates to the possibility of artificial increase in the number of neurons by means of the growth hormone preparation. These first experiments were performed on tadpoles; since tadpoles do not yet have a cortex in the meaning of a mammal's cortex, the entire cerebral hemispheres were investigated.

The term "growth hormone preparation" as used hereinafter refers to above mentioned growth-promoting pituitary extract, regardless whether it exists as an actual specific "growth hormone" or merely as a fortunate combination of other hormones.

[1]The histological part of this work has been done in the Laboratory of Experimental Embryology and in the College of Pharmacy of Columbia University.

B. Literature

In man the period in which the prospective neurons still have the ability to proliferate ends in the 4th or 5th month of foetal life. In rats, mitoses in the cortex were found even 21 days after birth (Hamilton, '01; Allen, '12; Sugita, '18; Smith, '34). There is no literature dealing with the end of this period relative to amphibians, but what we do know is that even in the late larval period numerous mitoses of prospective nerve cells were found (Coghill, '33). That the nerve cells (or prospective nerve cells) still proliferate in the late larval period, is also confirmed by this investigation, since older groups have always had a higher number of nerve cells.

There is also no literature about the influence of the growth hormone on neuronal hyperplasia; as for the other organs, recent publication of Lee and Freeman ('40) who demonstrated the hyperplasia of liver cells in response to the growth hormone administration, should be cited. As far as the author is aware, the present investigation is the first in the field of influence of the growth hormone on the central nervous system *in the early stage*.

On the other hand, there are many papers concerning the increase in body weight in response to growth hormone administration in the foetal or larval stage.

As far as the mammals are concerned, the growth hormone has been injected into pregnant rats (Teel, '26; Hain, '34; Sontag and Munson, '34; Watts, '35) and has caused a surplus of weight of the foetal tissues up to 14 per cent. In birds, Pighini ('37) reported a stimulation of embryonic growth upon injection of fowl pituitary into incubating hen eggs.

Passing to the amphibians, the case of Hahn ('12) should be cited first. Hahn investigated tadpoles of very large dimensions (gigantism) caused by a pathological hypertrophy of their pituitary. These tadpoles have shown an increase in body length of about 3.3 times the normal length and about the same increase in the size of the brain. An examination of Hahn's results relating to brains permits us to assume that the tadpoles with hyperpituitarism had more neurons than normal tadpoles.

There are also many experiments in which tadpoles or other amphibian larvae were subjected to the additional influence of the growth hormone preparation or pituitary body. Gudernatsch ('15) fed tadpoles u.a. on pituitary gland and found accelerated growth rates. Smith ('18) found the prolonged period of growth and hence larger

final dimensions in tadpoles fed on pituitary as compared with the controls fed on liver. Uhlenhuth ('20, '21, '22, '23) obtained similar results with salamanders after metamorphosis; the animals fed on hypophyses were much bigger and heavier than the controls fed on liver or worms and also bigger and heavier than all known animals of this species. Smith and Smith ('22, '23) stated that the intraperitoneal injections of extract of the anterior pituitary lobe cause the retardation of metamorphosis in axolotls, and this protracted larval state may produce gigantism in the animal. They found that the tadpoles which have been injected with pituitary extract may grow nearly twice as long as the normal ones. The stimulation of growth of tadpoles (hypophysectomized) through injections of anterior pituitary extract was found also by Evans ('24). Krizenecky ('24) and Krizenecky and Podhradsky ('26) added different growth hormone preparations to water in which tadpoles were kept, or fed tadpoles on pituitary, and always found stimulated growth in the animals so treated. Burns ('31) injected intraperitoneally axolotls with alkaline extract of the anterior lobe of mammalian pituitary or transplanted pituitary tissue, and found "a conspicuously enlarged head" in the experimental animals. Belkin ('34) added the growth hormone extract to water in which axolotls where kept, and found an appreciable increase in the speed of regeneration (i.e., also an increase in the speed of cell proliferation) in experimental animals over controls. Similar results were also obtained by Herrell ('34) with the growth hormone preparation Antuitrin *G* added to the water in which larvae of Rana clamitans were kept. He found a "striking increase in body size" and an increase in the size of the eyes. The last mentioned growth hormone preparation (Antuitrin *G*) was also used by the author in the present investigations.

C. Materials and Methods

Tadpoles of Rana pipiens from the vicinity of New York were divided into eight groups, so that in each pair of groups (one control group and one experimental) there were tadpoles of approximately the same mean age, weight, and size, although the tadpoles of the control groups were at the beginning slightly larger and heavier than experimentals; as far as the weights are concerned, the reverse was obtained in some groups after injections of growth hormone. In each successive pair of groups the tadpoles were older but they were always before metamorphosis; the tadpoles in the four younger groups had

no limbs until the end of the experiment; in the four older groups they had small hind limbs at the beginning, as well as fore limbs at the end of the experiments.

The groups were kept in identical glass jars together with water plants which were readily eaten by the tadpoles; the water was changed every day. The light, temperature, and feeding (dried spinach, bread, and water plants) were identical in all groups.

The tadpoles of the experimental groups were injected intraperitoneally every other day, each with 0.05 to 0.2 cc. (0.5 to 2.0 rat units) of growth hormone preparation Antuitrin *G* (Parke, Davis & Co.). The injections were made with needle 27 gauge and 1 cc. syringe. It should be remarked that not a full dose remained in peritoneum, but a part of it escaped through the opening, after withdrawal of the needle. The data concerning the experiments are given in Table 1.

At the end of the experiments the tadpoles were sacificed, fixed in Bouin's fluid and then preserved in 70 per cent alcohol until sectioning. The sections of seven micra were prepared by means of the paraffin method, stained with Delafield Haematoxylin and counterstained with eosin or light green. From serial sections in the groups to be compared (E_1—C_1, E_2—C_2, E_3—C_3, E_4—C_4) the sections at the same level were chosen; these sections were situated in the precomissural region, slightly anterior to foramen of Monro (Ecker and Wiedersheim, 1899; Kappers and Hammer, '18; Kappers, Huber, and Crosby, '36). Both pars pallialis and subpallialis have been taken into consideration.

The slides were observed with a Spencer microscope under total magnification 100×; in more crowded places (ventricular), a magnification 440× was used. The counting of the number of cells (nuclei), as well as calculation of areas of the sections, were performed by means of a Spencer reticule 5 mm. square ruled into 100 0.5 mm. squares, inserted in the eyepiece of the microscope; the number of cells in each of 100 small squares were calculated and then added. All together about 317 thousand cells were counted, which took about two months.

Since the object of the investigation was to compare the number of cells in injected and control animals, all the cells were counted, disregarding the fact that not all of these cells were or would become neurons; in such young brains it is sometimes impossible to discern non-nervous cells. Besides, the parts of the cells cut without nuclei were not counted in either experimental or control animals. These

TABLE 1

DESCRIPTION OF THE TADPOLES USED

Group	Final number of animals in group	Dates of experiments 1940	Mean dimensions at the beginning of the experiment			Mean final dimensions			Number of injections per animal	Doses per animal cc.
			Total length mm.	Body length mm.	Wet weight grams	Total length mm.	Body length mm.	Wet weight grams		
E_1	5	April 20-	30.73	11.6	0.4	32.3	11.6	0.484	7	0.05-0.1
C_1	5	May 3	30.9	12	0.4	33.6	12	0.408	0	———
E_2	6	April 20-	35.25	13.2	0.728	40	14.1	0.867	6	0.05-0.1
C_2	8	May 2	37.15	13.5	0.743	41	13.6	0.756	0	———
E_3	13	May 1-	82.6	28.8	3.88	84.3	29	6.6	11	0.15-0.2
C_3	8	May 28	84.1	29	3.98	84.4	29	7.4	0	———
E_4	18	May 1-	94	31.8	4.85	94	31.8	7.0	11	0.15-0.2
C_4	9	May 29	93.5	31.7	5.17	94	31.8	7.92	0	———

methods which were adapted also by Sugita ('17, '18) are quite satisfactory for comparison of experimental animals with controls, although not exact, if absolute number of cells is to be precisely measured.

The significance of the mean values of all groups has been investigated by means of the statistical "*t*-test" method, described in the text-books by Fisher ('38) and Mainland ('38) for small groups. Besides the significance of the results, the ratios of section areas and the ratios of the number of cells in experimental over control animals have been calculated. To obtain the ratio of the entire volumes of the hemispheres the ratio of areas was raised to the $\frac{3}{2}$ power, since it can be assumed that the increase is similar in all sections or in all three dimensions.

To obtain the ratio of number of cells in the entire volume the ratio of number of cells in sections of the same thickness was multiplied by the square root of the ratio of areas; this square root denotes the ratio of the lengths of the brain perpendicularly to the surface of the section, since it is again assumed that the increase in brain dimensions is similar in all three dimensions.

D. Results

The results of the experiments are represented in Tables 2 and 3 and in the corresponding diagram, Figure 1.

In Columns 3-6 (Table 2) are given the areas and numbers of cells as counted in both hemispheres of each section. In Columns 2 and 3 (Table 3), Probability P or P' denotes the possibility that the same mean result could have been obtained by random sampling of the tadpoles. These possibilities as well as standard error in Table 2 have been calculated by means of the forementioned "*t*-test" statistical method. The results in Table 3 mean that there is very small chance that the result could have been obtained also without any treatment. According to the standard evaluation of this probability (Mainland, '38), the result of the experiments should be called *significant* and in some groups *highly significant for areas of sections* (with exception of Group 2), and *highly significant for number of cells in sections of all groups*.

In Column 4, Table 3, R_a means the ratio of section areas of control and experimental animals. In Column 5, R'_a denotes the ratio of number of cells in corresponding sections of control and experimental animals.

Next columns give the ratio of volumes or the ratio per volume of

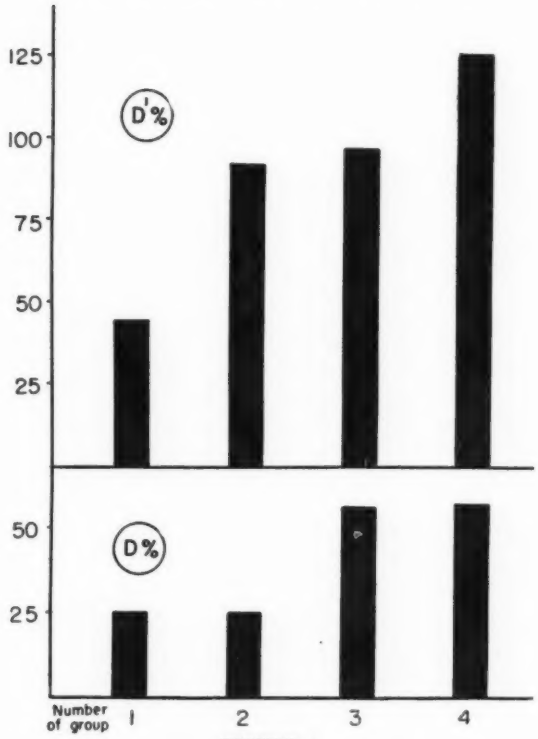

FIGURE 1

D'—Surplus of the number of neurons caused by the injections of the growth hormone, in per cent, per entire volume of cerebral hemispheres.
D—Surplus of the volume of cerebral hemispheres caused by the injections of growth hormone, in per cent.

hemispheres R_v and R'_v (Columns 6 and 7). The difference (R_v—1) or (R'_v—1), expressed in per cent, denotes the surplus of volumes or the surplus of nerve cells per volume of hemispheres over the control animals (Columns 8 and 9). This surplus is as high as 25.2 to 58.9 for volumes of cerebral hemispheres, and 44 to 126 for numbers of cells by volume of cerebral hemispheres.

Figures 2 to 9 represent the microphotographs of some of the typical sections of each of the groups to be compared. The surplus of areas and hyperplasia of cells in injected animals are pronounced.

TABLE 2

Group	Number of individual tadpole	Area of section of both hemisph. sq. mm. For each tadpole	Area of section of both hemisph. sq. mm. Mean and standard error	Number of cells per section in both hemisph. For each tadpole	Number of cells per section in both hemisph. Mean and standard error
	1	1.048		3596	
	2	1.148		3572	
E_1	3	0.894	1.075	2832	3410
	4	1.120	±0.0485	3478	±129
	5	1.036		3260	
	6	1.202		3708	
	1	0.858		2562	
	2	1.106		2770	
	3	0.770		2164	
C_1	4	1.090	0.916	3178	2560
	5	0.804	±0.0419	2308	±108
	6	0.835		2422	
	7	0.934		2430	
	8	0.928		2664	
	1	1.010		4538	
	2	0.970		4552	
E_2	3	1.215	1.096	5698	4696
	4	1.275	±0.0504	4820	±221.8
	5	1.010		3872	
	1	1.040		3248	
	2	0.886		2558	
C_2	3	1.060	0.944	2726	2642
	4	1.038	±0.0502	2392	±204
	5	0.770		2346	
	6	0.864		2618	
	1	2.07		5390	
	2	1.49		4681	
	3	1.83		4754	
	4	2.09		4982	
	5	2.23		4436	
	6	2.125	2.02	6298	5600
E_3	7	2.615	±0.0695	6890	±188.8
	8	1.855		5778	
	9	2.34		6242	
	10	1.76		5250	
	11	1.68		6108	
	12	2.005		5372	
	13	2.125		6552	
	1	1.79		3930	
	2	0.80		2374	
	3	1.80		3368	
C_3	4	1.50	1.530	3274	3278
	5	1.51	±0.0884	3392	±240
	6	1.46		3194	
	7	1.58		3576	
	8	1.80		3094	

TABLE 2 (*continued*)

Group	Number of individual tadpole	Area of section of both hemisph. sq. mm. For each tadpole	Area of section of both hemisph. sq. mm. Mean and standard error	Number of cells per section in both hemisph. For each tadpole	Number of cells per section in both hemisph. Mean and standard error
	1	2.04		5440	
	2	2.18		5566	
	3	2.02		5516	
	4	2.13		6512	
	5	2.18		5746	
	6	2.38		5626	
	7	2.43		5194	
	8	2.51		5924	
E_4	9	2.13	2.24	6806	6150
	10	1.89	±0.0488	5200	±141
	11	2.195		6961	
	12	2.385		7242	
	13	2.43		6854	
	14	2.25		6822	
	15	2.075		5808	
	16	2.45		7260	
	17	2.5		6346	
	18	2.14		5822	
	1	1.82		3438	
	2	1.7		3490	
	3	1.76		3026	
	4	1.71	1.665	3470	3171
C_4	5	1.92	±0.069	3348	±200
	6	1.755		3248	
	7	1.49		3164	
	8	1.83		3380	
	9	1.00		1988	

E. Discussion

Table 3 and the diagram in Figure 1 show that the growth hormone has produced a surplus of nerve cells as high as up to 126 per cent. The action of the growth hormone on embryonic, foetal, or larval tissue (Van Dyke, '36, p. 81) is a ***proliferation*** action until the progressing differentiation makes the mitoses impossible (as generally recognized there is an antagonistic action of proliferation and differentiation).

Further study of Tables 2 and 3 reveals the following. The influence of the growth hormone on hyperplasia of nerve cells is much more accentuated than on hyperplasia of other cells or on total weight of the body. This result conforms with the author's opinion expressed in the first paper, that cells such as neurons which at an early stage loose their ability to divide because of an early differentiation, should

FIGURE 2

CEREBRAL HEMISPHERES OF THE TYPICAL TADPOLE OF INJECTED GROUP E^1
Tranverse section in precomissural region, slightly anterior to foramen of Monro. Paraffin method, sections 7—micra stained with Delafield Haematoxylin and counterstained with Eosin. x100. The areas of sections, and numbers of cells obviously higher than in the control animal below.

FIGURE 3

CEREBRAL HEMISPHERES OF THE TYPICAL TADPOLE OF CONTROL GROUP C^1
Sectioned and stained as above. The same magnification.

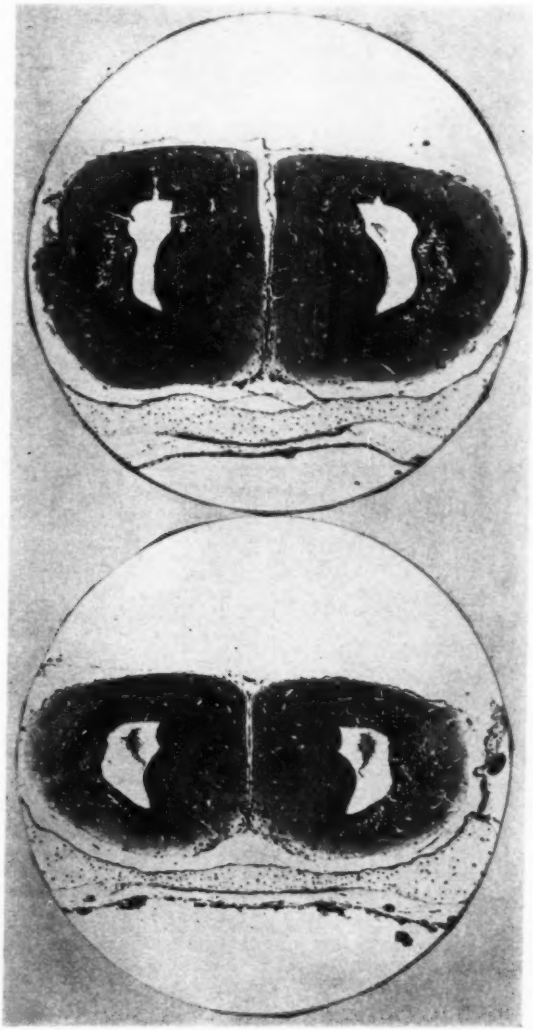

FIGURE 4

CEREBRAL HEMISPHERE OF THE TYPICAL TADPOLE OF INJECTED GROUP E^2
Transverse section in precomissural region, slightly anterior to foramen of Monro. Paraffin method, sections 7—micra stained with Delafield Haematoxylin and counterstained with Light Green. x50. The areas of sections, and numbers of cells obviously higher than in the control animal below.

FIGURE 5

CEREBRAL HEMISPHERES OF THE TYPICAL TADPOLE OF CONTROL GROUP C^2
Sectioned and stained as above. The same magnification.

FIGURE 6

CEREBRAL HEMISPHERES OF THE TYPICAL TADPOLE OF INJECTED GROUP E^3
Transverse section in precomissural region, slightly anterior to foramen of Monro, Paraffin method, sections 7—micra stained with Delafield Haematoxylin and counterstained with Light Green. x45. The areas of sections, and numbers of cells obviously higher than in the control animal below.

FIGURE 7

CEREBRAL HEMISPHERES OF THE TYPICAL TADPOLE OF CONTROL GROUP C^3
Sectioned and stained as above. The same magnification.

be much more susceptible for the proliferation stimuli (growth hormone) acting within specified time, than are the other cells with a long proliferation period for the stimuli acting within the same specified time, because the period of stimulation is relatively longer in the former cells than in the latter.

It should be further remarked that the increase in the number of cells per area was higher than the increase in area itself, and that, be-

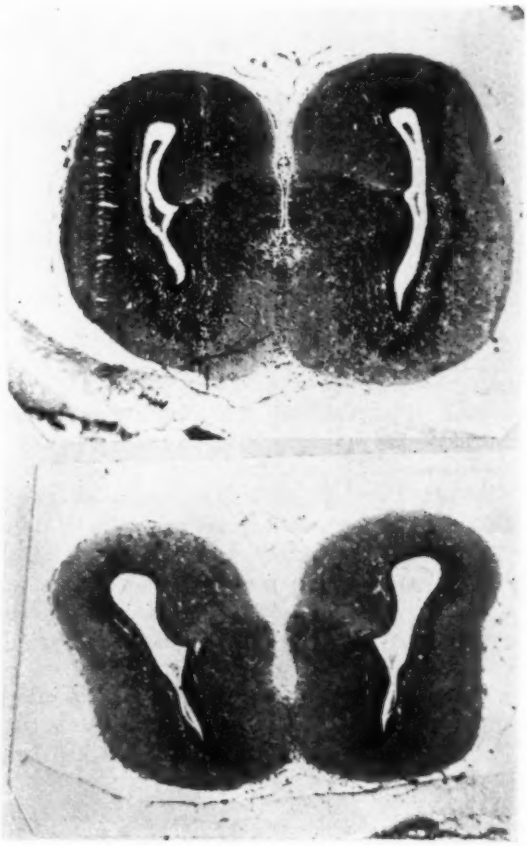

FIGURE 8

CEREBRAL HEMISPHERES OF THE TYPICAL TADPOLE OF INJECTED GROUP E^4
Transverse section in precomissural region, slightly anterior to foramen of Monro, Paraffin method. sections 7—micra stained with Delafield Haematoxylin and counterstained with Light Green. x45. The areas of sections, and numbers of cells obviously higher than in the control animal below.

FIGURE 9

CEREBRAL HEMISPHERES OF THE TYPICAL TADPOLE OF CONTROL GROUP C^4
Sectioned and stained as above. The same magnification.

cause in the injected animals the prospective neurons were probably smaller and more crowded than in the controls. This means that in the injected animals these cells are retained longer in a younger

TABLE 3

Group	Probability P for areas	Probability P' for number of cells	Ratio R_a of mean areas for injected and control animals	Ratio R'_a of mean number of cells in section	Ratio R_v of volumes for injected and control animals	Ratio R'_v of number of cells in entire volume of cerebral hemispheres for injected and control animals	Difference of volumetric ratio $(R'_v-1)100=D$ per cent	Difference of volumetric ratio of number of cells $(R'_v-1)100=D'$ per cent
E_1-C_1	0.025 (Significant)	0.0002 (Highly significant)	1.162	1.331	1.254	1.44	25.4	44
E_2-C_2	0.055 (Non-significant)	Less than 0.0001 (Highly significant)	1.161	1.775	1.252	1.92	25.2	92
E_3-C_3	0.00075 (Highly significant)	Less than 0.0002 (Highly significant)	1.32	1.71	1.515	1.965	51.5	96.5
E_4-C_4	Less than 0.0001 (Highly significant)	Less than 0.0001 (Highly significant)	1.36	1.94	1.589	2.26	58.9	126

(embryonal) state, thus being more able to proliferate; this result conforms with a well-known action of growth hormone to maintain the cells longer in the embryonal state; this means also that the ratios in Table 2 might be still higher if the animals had been allowed to reach maturity.

F. Conclusion

Evidence has been presented that growth hormone preparation when injected intraperitoneally into the tadpoles caused an increase in number of nerve cells as high as up to 126 per cent as compared with the controls.

G. Summary

This paper is the first in a series dealing with experimental investigation of the theory outlined in a previous paper, that by means of the growth hormone it may be possible to stimulate artificially the proliferation of prospective cerebral neurons, in an attempt to increase the higher functions of the brain.

Tadpoles, of different age groups, were subjected to intraperitoneal injections of the growth hormone preparation Antuitrin *G*. After sectioning the number of neurons, or prospective neurons, in the vicinity of the foramen of Monro were counted. These numbers as calculated for the entire cerebral hemispheres were 44 to 126 per cent higher in injected animals than in the controls. These differences according to statistical nomenclature are highly significant. It is therefore concluded that by means of the growth hormone it is possible to increase artificially and deliberately the number of brain neurons, at least in amphibians.

Acknowledgment

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Growth, 1941, **5**, 141-147.

A CURVE FOR MENTAL GROWTH BASED ON OVERLAPPING BETWEEN AGE LEVELS

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In this paper, it is proposed that a curve of mental growth may be constructed on the basis of the degree of overlap between mental tests at successive age levels. By assuming that the later mental test score represents an increment in performance over the earlier test score, one may, by using r^2, consider that the trend line from r_{12} to $r_{22'}$, where r_{12} represents the correlation of the first and second tests, and $r_{22'}$ represents the self correlation of the second test. A fundamental assumption for this thesis is that mental growth is characterized by an increase in the number of things the child can do. This could mean that certain ability or abilities underlying these performances increase in quality or number.

It is clear that for the construction of a growth curve based on test correlations between age levels, it is necessary to have a group of children who were tested at several age levels. Studies of this type have been relatively few, but they have consistently demonstrated, I think, that correlations are greatest when the time interval between the tests is least, and lowest when the time interval is greatest. This is what one would expect, of course. The longer the intervening time, the greater is the opportunity for environmental influences to shift the standing of individual children.

Using the concept of covariance, then, we could say, generally speaking, that the proportion of performance at a given later age level is present in decreasing amount as one goes back to the earlier and still earlier test. Let us assume, for example, that the correlation between Binet tests at eight and twelve years is .75. Then $(.75)^2$, or .56, would represent the proportion of the twelve-year test ability present at eight years. The six-year test might correlate .60 with the twelve-year test; its proportion of twelve-year variance would then be $(.60)^2$ or .36. On such a basis we could draw a curve to represent the increase in twelve-year-old Binet performance from six to eight years. The slope would be from .36 to .56 over this period. It we assumed the self-correlation of the twelve-year test to be, say

.95, or even perfect—1.00—we could then draw our curve to the point at twelve years represented by the square of this self-correlation or reliability coefficient for the terminal test, and so have a curve from six to twelve years. Our curve would represent not the increase in Binet performance generally, but the increase in twelve-year Binet performance specifically.

I should like to point out here that a comparison between growth curves based on this idea of increasing covariance with the later test on the one hand and the increase in number of successful items on the other is a complicated proposition. The increasing covariance represents an increase in the similarity of the areas covered by the earlier and later tests. It does not mean necessarily that the six-year test, if it correlates .60 with the twelve-year test provides, therefore, evidence that the child's mental age at six is 36 per cent of the mental age at 12. It means that the areas measured by the two tests have 36 per cent in common; we are simply attributing to the six months' test 36 per cent of the twelve months' area. This area is a matter of the complexity of performance as well as the amount.

It is clear that the correlation of the earlier tests with the later (terminal) test may be combined in multiples (R^2) to give the maximum degree of correlation of earlier test performance with the terminal performance.

In the accompanying figures, I have prepared curves based on three sets of data: (*a*) test results for 61 infants studied by Bayley (1933) during the first three years; (*b*) for two groups of children ($N = 104$, 108) given one of two preliminary forms of the California Pre-school Scale from about two to seven years, studied by Honzik (1938); and (*c*) for 40 children given the 1916 Stanford-Binet from six to eleven years by Baldwin and Stecher (1922).

In Figure 1 curves for r^2 and R^2 (the multiple coefficient, which includes all previous measures predicting the later measure) are presented the data of Bayley's study of infants. According to the slope of these curves, the acquisition of abilities to perform on the Bayley infant tests follows a straight line after about nine months. Before this point, however, there is a curve of positive acceleration. This finding is in accordance with the results obtained by Bayley herself and by Richards and Nelson (1939), which demonstrated that baby *mental* test material as one proceeds to the later age levels is composed of decreasing amounts of performance exogenous to the intellectual area.

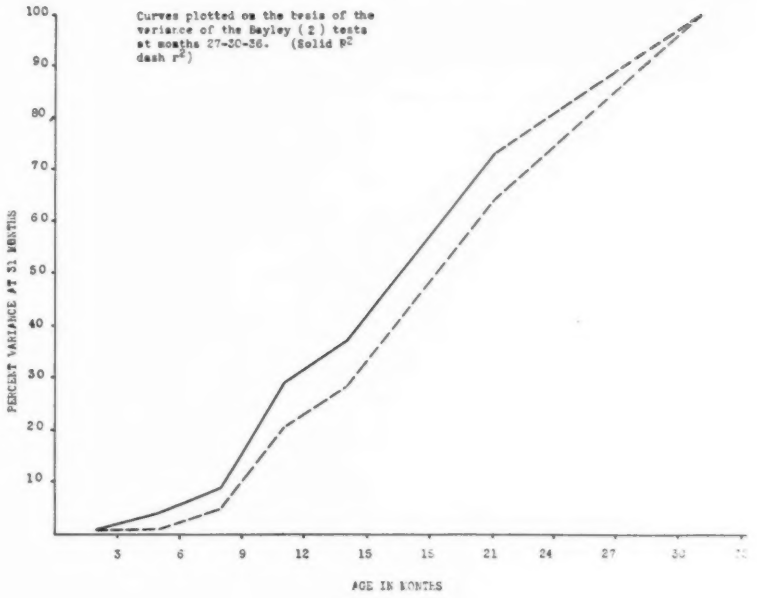

FIGURE 1

When the R^2 curve in Figure 1 is compared with the curve Thurstone calculated for Bayley's material, the relation shown in Figure 2 results. Due to factors of error in each curve it is possibly unwise to emphasize differences. But the greater difference occurs early in infancy.

The very procedure by which the two curves are calculated may account for this difference. Thurstone's technique is, of course, based wholly on relative variability between age levels; it embraces no concept of overlapping function. But the R^2 curve would be a straight line to the degree that there was an evenly stepped-up overlap between age levels. And, as pointed out in the preceding paragraph, evidence from other sources suggests that there is actually a difference in type of test item between the age levels from six months or less and those at a year or more.

In Figure 3 curves for the Honzik data have been drawn. These curves are based on correlations with the Stanford-Binet at six years.

In these Honzik curves it is interesting to note how little of the

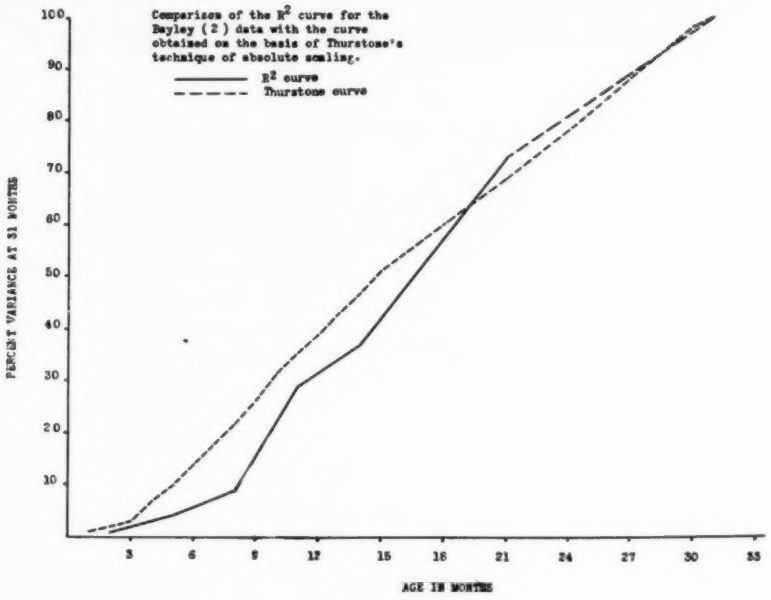

FIGURE 2

Stanford-Binet type of material is present at 21 months—10 per cent or less. The difference between the two Honzik curves is worth noting, also. The tests were reported to intercorrelate to the extent of .71. (The Cal. I test is most similar, according to Honzik, to the present form of the test.) Both curves show the period of most rapid acquisition of Binet-type ability to be from 22 to 42 months, after which the degree of acceleration lessens.

In Figure 4 curves for the Stanford-Binet material are presented. The solid curve represents the R^2 function based on the data of Baldwin and Stecher, the broken line the absolute scaling of Thurstone and Ackerson (1929) for their own group of cases. This comparison suggests that in terms of overlapping function children at six, seven, and eight years possess more of the ability required at ten years than Thurstone's curve would indicate. Of course, the differences between the groups could be due to sampling, since they are not identical groups. In fact, the Baldwin and Stecher group was composed of average and superior children, while the Thurstone Ackerson group

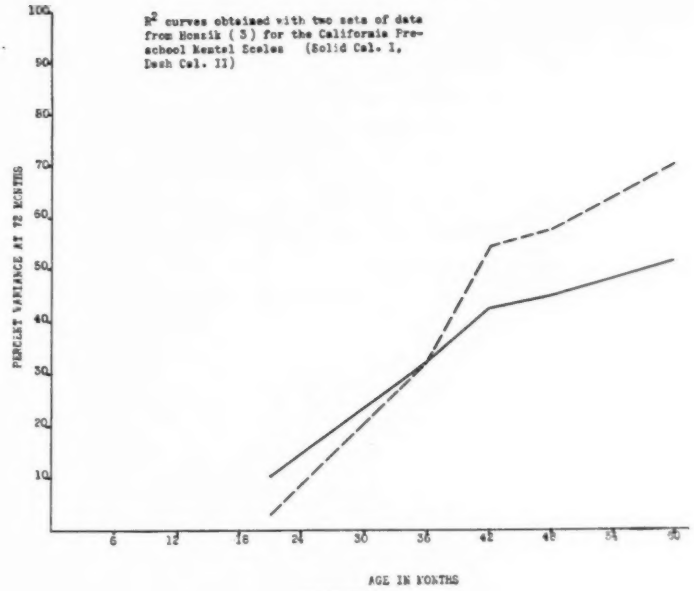

FIGURE 3

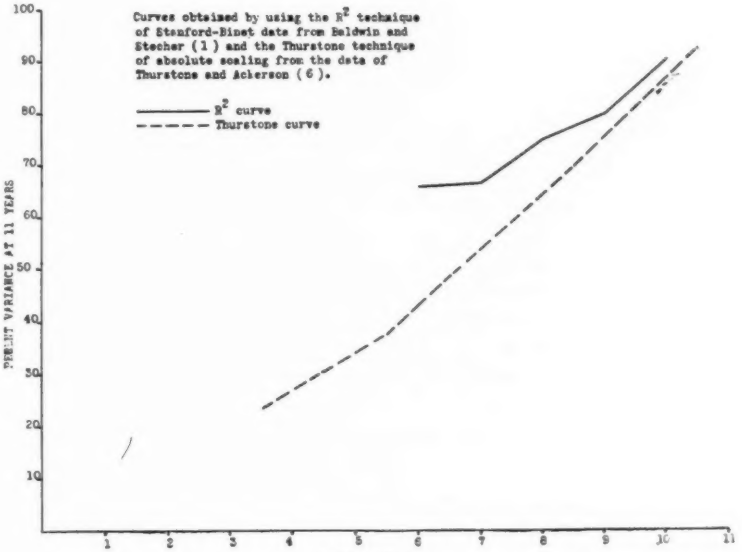

FIGURE 4

was composed of children with *IQ*'s largely below 100. But since the ordinate for each curve is determined by internal characteristics of the respective group (in one case relative variability, in the other terminal mental test), they are perhaps comparable.

Figure 5 presents a composite of the Bayley, Honzik, and Baldwin

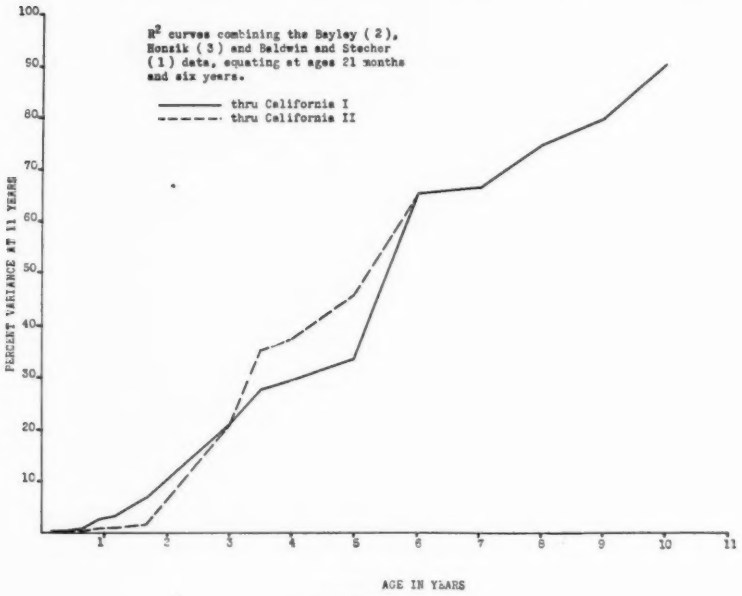

FIGURE 5

and Stecher data. It presumes that the terminal Bayley material (at 31 months) is similar in type of the California I and II material at that age level. Since Honzik's correlations used the Stanford-Binet as the terminal test, this assumption is not necessary for the second junction. But throughout, it is necessary to assume that the three groups of children represent similar samples.

If these assumptions are made for the curves in Figure 5, it can be seen that acquisition of the Binet type ability is most rapid from 1½ to six years, and that it is evidenced most clearly by the use of the California II type test through the period from two to six.

Summary

Employing the concept of covariance between age levels for mental tests, it is possible to construct curves for mental growth. In the case of Bayley's baby test material, such a curve was very similar in shape to Thurstone's curve (based on absolute scaling). For Honzik's data the curves suggested that the acquisition of Binet-type ability or abilities is very rapid from 22 to 42 months, and continues. For the data of Baldwin and Stecher, on older children, it was found that there was evidence at six years of considerably more of the ten-year-old performance than previous mental growth curves had indicated.

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THE INFLUENCE OF l-CYSTEINE SULFINIC ACID ON THE GROWTH OF SPONTANEOUS TUMORS IN THE MOUSE

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A. INTRODUCTION

Voegtlin (1940) in his introduction to the *Journal of the National Cancer Institute* states, "A different approach is to discover chemicals with a *specific* action on cancer cells. . . . The proposition is an extremely difficult one. . . . For the present and until we have a clearer conception of the cancer cell, efforts in this direction will have to be confined to empirical testing of compounds. . . .". He thus reiterates, and simply, a principle which has guided this work and that of preceding reports (Hammett, 1930).

What seemed to be potential getting away from empiricism was the finding that the partially oxidized derivatives of the naturally occurring chemical group sulfhydryl acted to retard cell increase in number (Hammett, 1930). This opened up the possibility that by attaching these proliferation inhibiting groups to appropriate radicals a compound might be obtained which would be of practical use in retarding the growth of cancer tissue (Hammett, 1930a). It was natural to make the first trials with derivatives of naturally occurring compounds. It was found that although the disulfoxide of cystine was a retardant of mouse tumor growth as of other expressions of proliferation (Staff of the Lankenau Hospital, 1936; and Lavine, 1935), its low solubility precluded extension of its use to any but theoretical studies. Attention was next given to a sulfoxide of methionine. This compound also proved to be only helpful from a theoretical point of view, since it appeared to be quite easily reducible and stimulative as well as retardative of mouse tumor growth (Hammett, 1941). Concurrently with these experiments, trials were run with l-cysteine sulfinic acid. This Sulfinic group, like the sulfoxide, has been found retardative of proliferation in lower forms (Hammett, 1930). This paper reports the results of this effort and compares them with the preceding.

B. Material and Methods

The l-cysteine sulfinic acid was prepared, isolated, identified, and purified by Dr. T. F. Lavine of The Institute Staff (1936, 1937). The compound is reasonably soluble and quite stable in acid solution and at pH 7.5. The compound was administered as the sodium salt in order to avoid corrosive effects of the pure acid. The solutions were prepared by adding the required amount of NaOH to make a 1.25 molar concentration of l-cysteine-Na-Sulfinate of pH 7.5. Each mouse received 0.1 cc. of this solution containing 0.125 millimols of the Na-salt daily by intra-scapular subcutaneous injection. Only the freshly prepared solutions which were free from contaminants were used. As usual, the Bar Harbor *"D"* strain of mice served as test material. There were 76 control and 65 test mice. The latter will hereinafter be referred to as the *CSA* mice. The two sets lived simultaneously under like conditions of diet, temperature, and illumination. The kind and frequency of records and measurements were the same as that described in previous papers as was the procedure in general (Staff of the Lankenau Hospital Research Institute, 1936).

C. Results and Comment

The mean body weight and the mean volume of first tumors of the *CSA* mice at the beginning of record did not differ to a significant degree from that of the controls (Tables 1 and 4). In these respects

TABLE 1

The Mean Body Weight in Grams of the Control and *CSA* Treated Mice

Mice with but	Initial		Maximum		Terminal	
	Control	*CSA*	Control	*CSA*	Control	*CSA*
1 Tumor	27.7	27.0	33.9	34.6	32.3	31.2
2 Tumors	28.6	25.4	35.6	33.2	32.8	30.8
3 Tumors	26.3	30.6	34.2	36.5	32.8	32.0
4 Tumors	27.3	25.4	31.9	31.2	29.1	30.0
All comb.	27.8±0.27	27.1±0.29	34.4±0.36	34.5±0.35	32.4±0.41	31.2±0.41

the two sets were essentially homogeneous. It was a fact, however, as shown in Table 3, that the mice used for *CSA* injection were somewhat older than the controls at the time of first tumor appearance. This may have conditioned the extent of reaction to *CSA*. The mean body weight of the *CSA* mice at exitus was essentially the same as that of the controls (Table 1). Therefore, it can be assumed that the factorial significance of body weight in tumor growth was the same in

both sets. This is supported by the fact that the two sets did not differ with respect to mean maximum body weight (Table 1).

Table 2 gives the number and percentage of mice with multiple

TABLE 2

THE NUMBER AND PERCENTAGE OF CONTROL AND *CSA* TREATED MICE WITH BUT ONE, TWO, THREE, AND FOUR TUMORS

Series	One tumor		Two tumors		Three tumors		Four tumors		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	42	55.3	24	31.6	8	10.5	2	2.6	76	100.0
CSA	43	66.2	13	20.0	8	12.3	1	1.5	65	100.0

tumors. Since 44.7 per cent of the control mice had multiple tumors while but 33.8 per cent of the *CSA* set were similarly afflicted, it would appear as if the sulfinic acid had acted to retard tumor productivity.

Table 3 gives the mean age in days at which the successive tumors appeared and the interval between. The data show that the time interval between the first and second tumors, and between the second and third tumors was greater in the *CSA* mice than in the control set. The consistency is marred only by the mice with four tumors. Since there were only two of these in the control and one in the *CSA* set, the significance of the inconsistency is somewhat invalidated. It would seem a fair assumption to conclude that the appearance of the multiple tumors was delayed by *CSA*.

The average longevity of the control mice is 378.3 ± 6.03 days. The *CSA* mice lived to be 395.4 ± 7.41 days old. The difference between the means divided by its probable error gives the figure 1.79. Thus the difference is not valid according to the statistical canons in common use. But the one- and two-tumor mice lived four days longer than their controls after first tumor recording: and the three-tumor mice lived nine days longer. Life at least was not shortened by *CSA* and there was hint of slight drift towards lengthening.

Table 4 gives the mean initial and maximum volumes of tumors. The data show that the maximum size of the first tumors in the *CSA* mice was less than the controls in three of the four series; that the maximum size of the second tumors was greater in two of the three series and less in one; that the maximum size of the third tumors was less in both series; and less in the case of the fourth tumors. Furthermore, when the mice are grouped according to number of tumors per mouse as shown in the columns to the right, it is found that the maximum size attained by tumors in the *CSA* mice was less in all

TABLE 3

THE MEAN AGE IN DAYS AT WHICH THE FIRST, SECOND, THIRD, AND FOURTH TUMORS APPEARED IN THE CONTROL AND *CSA* TREATED MICE TOGETHER WITH THE MEAN INTERVAL BETWEEN THE SUCCESSIVE TUMORS

Tumor succession:	First tumor Incidence		Second tumor Incidence		Second tumor Interval		Third tumor Incidence		Third tumor Interval		Fourth Tumor Incidence		Fourth Tumor Interval	
Mice with but	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*	Cont	*CSA*	Cont.	*CSA*	Cont.	*CSA*
One tumor	318	325	—	—	—	—	—	—	—	—	—	—	—	—
Two tumors	310	308	338	349	28	41	—	—	—	—	—	—	—	—
Three tumors	318	358	351	393	33	35	365	415	14	22	—	—	—	—
Four tumors	308	496	333	509	25	13	341	511	8	2	347	516	6	5
Weighted mean	316	328	341	373	29	38	360	425	13	19	347	516	6	5

TABLE 4

THE MEAN INITIAL AND MAXIMUM VOLUME OF THE TUMORS OF CONTROL AND *CSA* TREATED MICE

		Tumor volume in cubic centimeters									
		First tumors		Second tumors		Third tumors		Fourth tumors		Means	
Mice with but	Status—	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*
One tumor	Initial	0.214	0.204	——	——	——	——	——	——	0.214	0.204
	Maximum	9.302	8.831	——	——	——	——	——	——	9.302	8.831
Two tumors	Initial	0.185	0.119	0.223	0.141	——	——	——	——	0.204	0.130
	Maximum	7.107	7.231	2.341	1.589	——	——	——	——	4.724	4.410
Three tumors	Initial	0.103	0.086	0.120	0.118	0.129	0.133	——	——	0.117	0.112
	Maximum	6.303	4.794	2.493	3.272	0.796	0.652	——	——	3.197	2.906
Four tumors	Initial	0.060	0.087	0.095	0.212	0.095	0.175	0.201	0.075	0.123	0.137
	Maximum	2.910	0.518	0.670	5.396	0.385	0.322	3.257	0.164	1.805	1.600
Means	Initial	0.189	0.170	0.191	0.136	0.122	0.137	0.201	0.075		
	Maximum	8.12	7.89	2.28	2.37	0.71	0.62	3.26	0.16		

groups. Finally, when the 24 comparable pairs of *CSA* and control tumors, which result when the tumors are classed according to volume at beginning of record (not shown) are inspected, it is found that the maximum size of the *CSA* tumors was less than the controls in 15 instances: greater in 7, and equal in 2. From all this it would appear as if *CSA* acted to reduce the maximum volume attainable by the tumors with a fair degree of consistency.

Table 5 gives the mean number of days required for the attainment

TABLE 5

THE MEAN NUMBER OF DAYS REQUIRED TO ATTAIN MAXIMUM TUMOR VOLUME AND THE MEAN DAILY VOLUME INCREASE FROM BEGINNING OF RECORD TO MAXIMUM VOLUME OF CONTROL AND *CSA* TREATED MICE

Mice with but	First tumors		Second tumors		Third tumors		Fourth tumors		Means	
	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*	Cont.	*CSA*
					Days required					
One tumor	54.8	60.3	—	—	—	—	—	—	54.8	60.3
Two tumors	55.7	61.2	30.9	18.8	—	—	—	—	43.3	40.0
Three tumors	65.8	74.3	33.9	42.4	18.6	20.6	—	—	39.4	45.8
Four tumors	60.0	21.0	31.0	19.0	13.5	12.0	22.0	7.0	31.6	14.8
Means	56.4	61.6	31.5	27.4	17.6	19.7	22.0	7.0	—	—
					Daily increase in volume					
One tumor	0.166	0.143	—	—	—	—	—	—	0.166	0.143
Two tumors	0.124	0.116	0.069	0.077	—	—	—	—	0.097	0.097
Three tumors	0.094	0.063	0.070	0.074	0.036	0.025	—	—	0.067	0.054
Four tumors	0.048	0.021	0.019	0.273	0.021	0.012	0.014	0.013	0.026	0.080
Means	0.142	0.126	0.066	0.085	0.033	0.024	0.014	0.013	—	—

of maximum volume and the mean amount of tumor tissue added per day during the time between the first and maximum tumor volume measurement. The data show that (*a*) in three of the four sets of first tumors a longer time was required to attain maximum volume in the *CSA* mice than in the controls, and that less volume was added each day; (*b*) in the three sets of second tumors less time was required in two and more time in one, and that more tumor substance was added per day; (*c*) in the two sets of the third tumors, longer time was required in both and less tissue was added per day; (*d*) and that while the fourth tumor took less time to attain maximum volume, less substance was added each day. Furthermore, when the mice are grouped according to the number of tumors per mouse, as shown in the columns to the right, it is found that the time required to attain maximum tumor size tended to be longer in the *CSA* set than in the controls in the mice with one and three tumors, and shorter in those with two and four tumors. In addition, the amount of tumor tissue

TABLE 6

THE MEAN VOLUME IN CC. OF ALL FIRST, SECOND, THIRD, AND FOURTH TUMORS SEPARATELY AND COMBINED OF CONTROL AND *CSA* TREATED MICE ON SUCCESSIVE FIVE-DAY INTERVALS FROM BEGINNING OF RECORD OF EACH TUMOR

Age of tumor in days	Number of mice		Average volume		Number of mice		Average volume		Number of mice		Average volume	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
	First tumors				*Second tumors*				*All tumors*			
0	76	65	0.189	0.170	34	22	0.191	0.136	122	97	0.184	0.158
5	76	65	0.345	0.359	34	22	0.383	0.339	122	96	0.352	0.342
10	76	65	0.591	0.642	33	21	0.564	0.683	120	94	0.576	0.618
15	75	65	0.883	1.108	31	18	0.836	1.095	115	89	0.858	1.055
20	74	65	1.375	1.757	28	13	1.206	1.462	109	82	1.292	1.655
25	74	64	2.073	2.369	24	10	1.613	1.579	103	77	1.928	2.190
30	71	60	2.666	2.833	19	7	1.778	1.447	95	70	2.419	2.594
35	67	54	3.275	2.910	16	6	1.856	2.308	87	63	2.889	2.752
40	59	52	3.824	3.478	11	5	1.385	3.086	72	59	3.354	3.358
45	54	50	4.418	4.154	8	5	1.157	3.629	62	56	3.997	4.044
50	46	42	5.034	4.085	4	4	0.729	4.463	50	46	4.689	4.118
55	37	36	5.586	4.596	3	3	0.729	2.979	40	39	5.222	4.471
60	36	34	6.467	4.868	2	3	1.433	3.023	38	37	6.202	4.718
65	31	30	6.996	4.912	2	2	1.911	1.220	33	32	6.688	4.681
70	23	24	6.779	5.318	2	1	2.426	1.869	25	25	6.431	5.180
75	17	19	6.987	4.771	2	1	2.889	1.893	19	20	6.556	4.627
80	15	15	6.934	3.991	2	–	3.765	——	17	15	6.561	3.991
85	10	15	5.916	4.401					10	15	5.916	4.401
90	8	11	5.838	3.366					8	11	5.838	3.366
95	8	8	5.777	3.329					8	8	5.777	3.329
100	4	7	8.213	3.270					4	7	8.213	3.270
105	4	7	9.192	3.337					4	7	9.192	3.337
110	3	4	7.212	4.422					3	4	7.212	4.422
115	1	3	4.776	1.850					1	3	4.776	1.850
120	1	3	5.114	2.352					1	3	5.114	2.352
125	1	3	5.401	2.473					1	3	5.401	2.473
130	1	3	6.637	2.826					1	3	6.637	2.826
135	1	3	7.363	3.644					1	3	7.363	3.644
140	–	3	——	4.170					–	3	——	4.170
145	–	3	——	4.437					–	3	——	4.437
150	–	3	——	4.305					–	3	——	4.305
155	–	3	——	4.033					–	3	——	4.033
160	–	2	——	2.513					–	2	——	2.513
165	–	1	——	4.098					–	1	——	4.098

TABLE 6 (*continued*)

	Control	Treated	Control	Treated	Control	Treated	Control	Treated
			Third tumors				*Fourth tumors*	
0	10	9	0.122	0.137	2	1	0.201	0.075
5	10	8	0.315	0.242	2	1	0.290	0.146
10	9	7	0.437	0.274	2	1	0.820	0.164
15	7	6	0.425	0.367	2	–	1.789	——
20	6	4	0.386	0.612	1	–	3.002	——
25	4	3	0.482	0.427	1	–	4.559	——
30	4	3	0.455	0.497	1	–	4.910	——
35	4	3	0.562	0.783				
40	2	2	0.315	0.932				
45	–	1	——	0.628				

added per day tended to be (*a*) less in the one- and three-tumor mice; (*b*) the same in the two-tumor sets; (*c*) and more in the four-tumor group. If the fourth tumors and the four-tumor mice are omitted because they were too few in number to be valid indices of difference, the assumption may be made that *CSA* retarded the attainment of maximum volume, and held up the amount of tumor tissue added per day. Consistent with these data is the architectural appearance of the *CSA* tumor sections. These, like those from the cystine disulfoxide mice (Staff of the Lankenau Hospital Research Institute, 1936), were less compact than the controls. Furthermore, more of the tumors

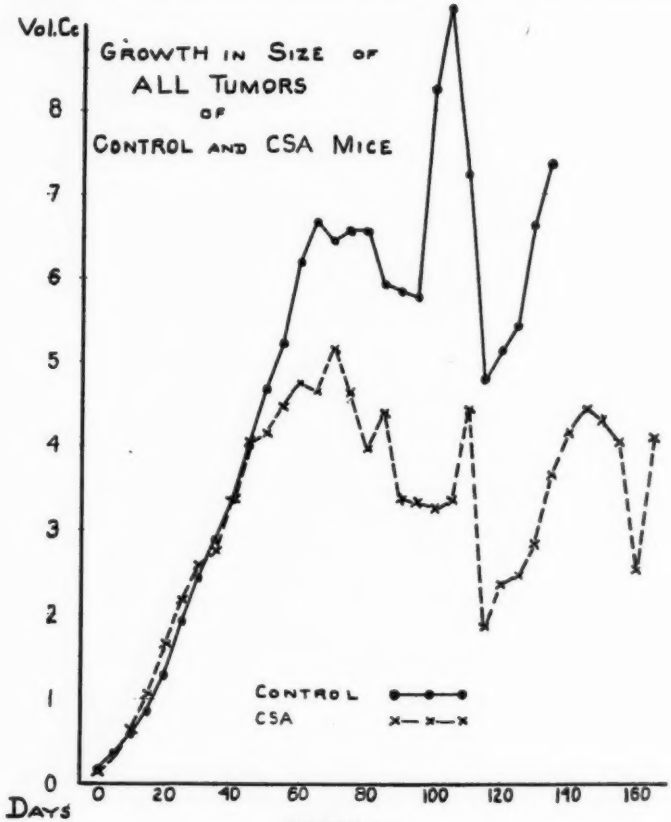

FIGURE 1

were composed of small pre-acinar clumps containing from four to eight nuclei. Since differences of this nature have already been recorded by pictures and counts, there would seem to be no need for repetition here. Suffice it to say that by and large tumor sections give evidence that cell increase in number was retarded. From this it may be concluded that the lesser size growth of the *CSA* tumors is attributable in large measure to this reaction.

Table 6 contains the mean volumes for the successive five-day intervals from beginning of record to exitus of all first, second, third, and fourth control and *CSA* tumors together with the number of tumors contributing to the means. In addition there is tabulated the means and numbers of all tumors combined. This latter record is visualized in Figure 1. It shows that tumor growth as a whole was held in check by the sulfinic acid. This retardation was reasonably consistent. Evidence of this is given in Table 7. This table

TABLE 7

THE CONSISTENCY WITH WHICH THE *CSA* TUMORS DEVIATED FROM CONTROL ON SUCCESSIVE FIVE-DAY INTERVALS FROM BEGINNING OF RECORD TO EXITUS

Mice with but	First tumors			Second tumors			Third tumors			Fourth tumors			Totals		
	+	—	=	+	—	=	+	—	=	+	—	=	+	—	=
One tumor	4	15	3	—	—	—	—	—	—	—	—	—	4	15	3
Two tumors	4	21	2	3	5	1	—	—	—	—	—	—	7	26	3
Three tumors	3	16	0	11	3	1	2	5	1	—	—	—	16	24	2
Four tumors	6	0	0	3	0	0	0	2	1	0	2	0	9	4	1
Totals	17	52	5	17	8	2	2	7	2	0	2	0	36	69	9
Combined	4	21	2	9	4	2	3	4	1	0	2	0	16	31	5

records the consistency with which the mean volume of the *CSA* tumors was greater or less than control by ten or more per cent when the data of Figure 1 are broken down into their constituent elements. This shows that retardation tended to be more or less general.

To summarize: (*a*) There were fewer mice with multiple tumors; (*b*) there was delay in appearance of multiple tumors; (*c*) the maximum size attained by the tumors was less and there was a trend towards delay in attainment of maximum size, as well as a trend towards a lesser daily increment in tumor tissue from beginning of record to attainment of maximum size; (*d*) the over-all tumor volume was less than control during a large part of tumor growth from beginning of record to exitus, and this was sustained with reasonable consistency in the breakdown analysis of the totals; (*e*) the tumors were less compact; (*f*) there was a slight drift to life extension;

(*g*) and body weight was essentially unaffected. The picture as a whole is consistent. It gives evidence that the administration of l-cysteine sulfinic acid produced a mild but definite retardation of mouse tumor growth without toxic action on the organism as a whole.

D. Discussion

It is pertinent briefly to compare the reaction of l-cysteine sulfinic acid with that to l-cystine disulfoxide (Staff of the Lankenau Hospital Research Institute, 1936), and to dl-methionine sulfoxide (Hammett, 1941). Without going into detail the principal points of difference are these. The retardation produced by sulfinic acid was neither as extensive nor as consistent as that evoked by disulfoxide. This is shown particularly in the secondary tumors where consistent retardation occurred in the former and less consistently in the latter. Furthermore, the life prolongation was clear-cut and definite in the case of the disulfoxide mice, while it was only suggested in the sulfinate. In comparing sulfinic acid with methionine sulfoxide, it is seen that the former was a more effective retardant than the latter. This is shown by the fact that the methionine derivative acted to accelerate growth of both second and third tumors, while the sulfinate had no such action. Interestingly enough in this connection is the fact recorded by Bennett (1937) that whereas dl-methionine sulfoxide may replace l-cystine in the diet of rats and act as a forwarder of growth, l-cysteine sulfinic acid had no such ability. Consistent with this expression of difference is the fact that the methionine sulfoxide tumor mice reached a greater maximum body weight and were heavier at exitus than the controls, while the cysteine sulfinate mice were no different from the controls in this respect.

Roughly speaking, then, it would appear that the effectiveness of l-cysteine sulfinic acid as a retardant of mouse tumor growth lies somewhere between that of l-cystine disulfoxide and dl-methionine sulfoxide.

It is appropriate to suggest why this should be so. It may be that the sulfinic acid is a lesser retardant than the disulfoxide either because it has but one partially oxidized group while the disulfoxide has two; or because its single sulfur atom is more oxidized than either of the two sulfur atoms of the disulfoxide and hence more liable to be removed from influence on proliferation by completion of its oxidation through the processes of living. In the one case it would be the difference in number or concentration of partially oxidized groups

which would be the basis of the difference in influence; in the other case it would be the stage of oxidation of the sulfur atoms which would be responsible. Both might participate.

Turning now to the basis of the difference between l-cysteine sulfinic acid and dl-methionine, the only reason I can think of is the probability that the former is less subject to reduction by tissue activities than the latter. If this suggestion is well taken, two factors might participate. The first is the more advanced state of oxidation of the sulfur in sulfinate. This might well make its reduction to inhibition in effectiveness more difficult. The second is the difference in the nature of the radicals to which the two partially oxidized groups are attached. The detailed exposition from which these suggestions derive has been given in previous papers (Hammett, 1930, 1941) and need not be repeated here. Suffice it to state that much more work must be done before answers can be given. All that the foregoing is intended to do is to point out lines of approach which may be fruitful.

E. Summary and Conclusions

Sixty-five female mice with spontaneous tumors were given daily non-toxic subcutaneous intrascapular injections of 0.125 millomols pure l-cystine Na-Sulfinate in 0.1 cc. distilled water at pH 7.5. Their tumor productivity and growth, body weight, and survival from the time of first tumor appearance to exitus was compared with the 76 controls observed simultaneously under like conditions of diet and environment.

It was found that whereas body weight growth was unaffected, tumor growth was definitely held back. (*a*) There were fewer mice with multiple tumors; (*b*) the appearance of secondary tumors was delayed; (*c*) maximum tumor size was less; (*d*) the time required to reach this tended to be lengthened; (*e*) tumor volume fell below that of the controls consistently over the greater part of the growth span; (*f*) and there was a slight drift to increase longevity.

It is concluded that l-cysteine sulfinic acid acts to retard the growth of spontaneous tumors in the mouse. Presumably it does this through the possession of a partially oxidized SH group which acts to hold back cell increase in number.

The retarding effectiveness seems to lie somewhere between that of cystine disulfoxide and methionine sulfoxide. Suggestions are given in the text as to why this should be so.

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A METHOD OF PLANNING FOR BASIC BIOLOGICAL RESEARCH; WITH ESPECIAL REFERENCE TO CANCER RESEARCH

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A. THE FORMAL STEPS

Organized coördinated research is an essential prerequisite for understanding of the complex and interrelated activities of living things. If confusion is not to be worse confounded, some method of planning must be evolved which would allow the tracing of the interplay of the several aspects of living in the determination of the answer to the biological problems which present themselves. The method here outlined is offered as a beginning in that direction. Undoubtedly it is not perfect. But no human effort attains perfection. It does however provide a basis for trial and discussion. Perhaps it will be found useful by those who wish to bring order out of chaos.

The first step in any contemplated research is to state the problem in the form of a simple question. This is the Key question.

The next step is to ask the Primary questions. These are three in number. They concern the Origin, the Behavior, and the End Result of the thing or process which is to be studied.

The third step is to integrate the Primary question with the basic Aspects of Existence: the Genetics, the Chemistry, the Physics, and the Anatomy. From this result the Secondary Questions. These are twelve in number.

The fourth step is to integrate the basic Aspects of Existence with each other and the Secondary Derivatives. From this come the Tertiary Questions. These are forty-eight in number.

The next step is to decide which one of the forty-eight Tertiary questions one wishes to find the answer for; and then go to work.

It is obvious that the chosen question can and should be broken down into others, most of which will be concerned with procedure rather than with inquiry into biological activity.

In order to demonstrate the working of this method of planning

I have chosen a question which we have set ourselves in the field of cancer research. The thoughtful reader will see how in getting the answer to this question, it is necessary to integrate the inquiry through the use of the basic sciences of genetics, chemistry, physics, and anatomy (1); and that for this, organized coördinated research is an essential prerequisite.

1. *Key Question*

KQ. How does a cancer cell differ from a normal cell?

2. *Primary Questions*

PQO. What is the Origin of the cancer cell?
PQB. What is the Behavior of the cancer cell?
PQER. What is the End Result of the cancer cell?

3. *Secondary Questions*

SQOG. What is the Genetics of cancer cell origin?
SQOC. What is the Chemistry of cancer cell origin?
SQOP. What is the Physics of cancer cell origin?
SQOA. What is the Anatomy of cancer cell origin?

SQBG. What is the Genetics of cancer cell behavior?
SQBC. What is the Chemistry of cancer cell behavior?
SQBP. What is the Physics of cancer cell behavior?
SQBA. What is the Anatomy of cancer cell behavior?

SQERG. What is the Genetics of cancer cell end result?
SQERC. What is the Chemistry of cancer cell end result?
SQERP. What is the Physics of cancer cell end result?
SQERA. What is the Anatomy of cancer cell end result?

4. *Tertiary Questions*

TQOGg. What is the Genetics of the genetics of cancer cell origin?
TQOGc. What is the Genetics of the chemistry of cancer cell origin?
TQOGp. What is the Genetics of the physics of cancer cell origin?
TQOGa. What is the Genetics of the anatomy of cancer cell origin?

TQOCc. What is the Chemistry of the chemistry of cancer cell origin?
TQOCg. What is the Chemistry of the genetics of cancer cell origin?
TQOCp. What is the Chemistry of the physics of cancer cell origin?
TQOCa. What is the Chemistry of the anatomy of cancer cell origin?

TQOPp. What is the Physics of the physics of cancer cell origin?
TQOPg. What is the Physics of the genetics of cancer cell origin?
TQOPc. What is the Physics of the chemistry of cancer cell origin?
TQOPa. What is the Physics of the anatomy of cancer cell origin?

TQOAa. What is the Anatomy of the anatomy of cancer cell origin?
TQOAg. What is the Anatomy of the genetics of cancer cell origin?
TQOAc. What is the Anatomy of the chemistry of cancer cell origin?
TQOAp. What is the Anatomy of the physics of cancer cell origin?

TQBGg. What is the Genetics of the genetics of cancer cell behavior?
TQBGc. What is the Genetics of the chemistry of cancer cell behavior?
TQBGp. What is the Genetics of the physics of cancer cell behavior?
TQBGa. What is the Genetics of the anatomy of cancer cell behavior?

TQBCc. What is the Chemistry of the chemistry of cancer cell behavior?
TQBCg. What is the Chemistry of the genetics of cancer cell behavior?
TQBCp. What is the Chemistry of the physics of cancer cell behavior?
TQBCa. What is the Chemistry of the anatomy of cancer cell behavior?

TQBPp. What is the Physics of the physics of cancer cell behavior?
TQBPg. What is the Physics of the genetics of cancer cell behavior?
TQBPc. What is the Physics of the chemistry of cancer cell behavior?
TQBPa. What is the Physics of the anatomy of cancer cell behavior?

TQBAa. What is the Anatomy of the anatomy of cancer cell behavior?
TQBAg. What is the Anatomy of the genetics of cancer cell behavior?
TQBAc. What is the Anatomy of the chemistry of cancer cell behavior?
TQBAp. What is the Anatomy of the physics of cancer cell behavior?

TQERGg. What is the Genetics of the genetics of the cancer cell end result?
TQERGc. What is the Genetics of the chemistry of the cancer cell end result?
TQERGp. What is the Genetics of the physics of the cancer cell end result?
TQERGa. What is the Genetics of the anatomy of the cancer cell end result?

TQERCc. What is the Chemistry of the chemistry of the cancer cell end result?
TQERCg. What is the Chemistry of the genetics of the cancer cell end result?
TQERCp. What is the Chemistry of the physics of the cancer cell end result?
TQERCa. What is the Chemistry of the anatomy of the cancer cell end result?

TQERPp. What is the Physics of the physics of the cancer cell end result?
TQERPg. What is the Physics of the genetics of the cancer cell end result?
TQERPc. What is the Physics of the chemistry of the cancer cell end result?
TQERPa. What is the Physics of the anatomy of the cancer cell end result?

TQERAa. What is the Anatomy of the anatomy of the cancer cell end result?
TQERAg. What is the Anatomy of the genetics of the cancer cell end result?
TQERAc. What is the Anatomy of the chemistry of the cancer cell end result?
TQERAp. What is the Anatomy of the physics of the cancer cell end result?

It has long been felt by some that the mathematics of engineering is not a complete system for the expression of attributes and relations which are peculiar to living things. An attempt is growing to develop a system whereby this difficulty may be surmounted. It is hoped that from this there will eventuate a system of biological mathematics which will have an expanding usefulness. The foregoing is a simple example of this attempt.

Now the foregoing is essentially a series of unsolved equations in which biological attributes are used instead of numbers. The answers to the equations derive of course from knowledge of the mutual interreaction of the biological attributes. These attributes are the factors of the equations. It is therefore mathematically necessary to set forth all the possible equations in which these factors may be presumed

to participate; and to derive and set forth the equations which indicate the possible direction of relations of the attributes.

A few of these questions may seem to yield an answer which in mathematical analysis is classed as an indeterminate. I have therefore drawn up what I consider is the basic meaning involved in the questions. In addition four diagrams are given from which the interrelations of the several attributes and derivatives may be visualized as well as the path of their contribution to the answer to the Key question.

B. The Exposition

1. *The "K" Designate*

KQ. 1. A cancer cell is a cell which produces a malignant growth.
2. A normal cell is a cell which does not produce a malignant growth.

2. *The "P" Derivatives*

PQO. The Origin of the cancer cell is concerned with the phylogeny and ontogeny of the cancer cell;
1. Where and how it appears on the evolutionary tree.
2. Where and how it comes from in the individual organism.

PQB. The Behavior of the cancer cell is concerned with the activity expressions of the cancer cell;
1. Its growth.
2. Its development.
3. Its metabolism.

PQER. The End Result of the cancer cell is concerned with the products of the cancer cell;
1. The cancer.
2. The effects of the cancer.

The Origin conditions the Behavior.
The Behavior conditions the End Result.
A part of the End Result is conditioned by the Origin alone; a part by the Origin through the Behavior.
These relations are visualized in Figure 1.

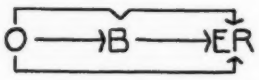

FIGURE 1
The Relations between Origin, Behavior, and End Result: The "*P*" Relations

3. *The "AE" Factors (Attributes)*

The chief Aspects of Existence are:

Direction (Continuance)	Substance	States of Substance	Form (Structure)
Genetics	Chemistry	Physics	Anatomy
G	*C*	*P*	*A*

Knowledge of these attributes is comprised in the four basic sciences which underlie them.
Genetics determines the substance (Chemistry).
Chemistry determines the state of substance (Physics).
State of substance determines structure (Anatomy).

Genetics determines the state of substance directly and by passage through substance.
Genetics determines structure directly and by passage through substance and state of substance.
Substance determines structure directly and by passage through state of substance.
Chemistry conditions expression of genetics.
Physics conditions expression of genetics directly and by passage through substance.
Physics conditions expression of chemistry.
Structure conditions expression of state of substance.
Structure conditions expression of chemistry directly and by passage through state of substance.
Structure conditions expression of genetics directly and by passage through state of substance and substance.
The "*AE*" Relations are visualized in Figure 2.

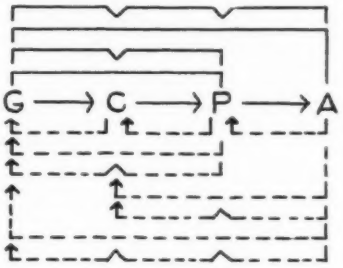

FIGURE 2
THE RELATIONS BETWEEN GENETICS, CHEMISTRY, PHYSICS, AND ANATOMY: THE "*AE*" RELATIONS

4. *The "SAE" Derivatives*

SQOG. The Genetics of cancer cell origin is concerned with the source of the strain of cells from which cancer cells are derived.
SQOC. The Chemistry of cancer cell origin is concerned with the substance of the cells comprising the cell strain from which cancer cells are derived.
SQOP. The Physics of cancer cell origin is concerned with the state of the substance of the cells of the cell strain from which cancer cells are derived.
SQOA. The Anatomy of cancer cell origin is concerned with the structure of the cells comprising the strain which gives rise to cancer cells.
SQBG. The Genetics of cancer cell behavior is concerned with the maintenance of cancer cells as cancer cells.
SQBC. The Chemistry of cancer cell behavior is concerned with the substance and changes of substance of cancer cells.
SQBP. The Physics of cancer cell behavior is concerned with the state of substance and changes in state of substance of cancer cells.
SQBA. The Anatomy of cancer cell behavior is concerned with the structure and form; and changes in structure and form of cancer cells.
SQERG. The Genetics of the cancer cell end result is concerned with the persistence of cancer cells as cancer cells.
SQERC. The Chemistry of the cancer cell end result is concerned with the substance of the cancer and its chemical effects.
SQERP. The Physics of the cancer cell end result is concerned with the state of substance of the cancer and its physical effects.
SQERA. The Anatomy of the cancer cell end result is concerned with the cytology and architecture of the cancer and its structural effects.

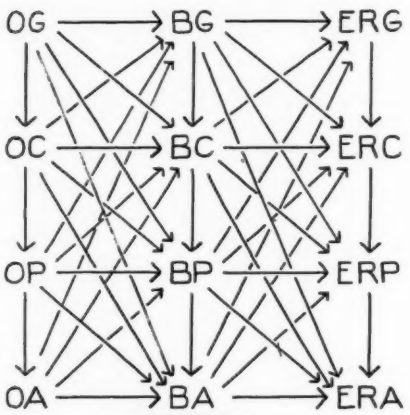

FIGURE 3

THF PATHS OF INTEGRATION OF THE CHIEF ASPECTS OF EXISTENCE WITH ORIGIN, BEHAVIOR, AND END RESULT: THE "*SAE*" RELATIONS

The "*SAE*" relations are visualized in Figure 3. In order to avoid confusion the lines showing the direct *O-ER* relations are omitted; as are the lines showing the conditioning relations existing among the *AE* factors. These can be derived by the reader from the preceding diagrams.

5. *The "TAE" Derivatives*

TQOGg. The Genetics of the genetics of cancer cell origin is concerned with the phylo- and ontogenetic derivation of the cancer cell.

TQOGc. The Genetics of the chemistry of cancer cell origin is concerned with the phylo- and ontogenetic derivation of the chemical groups peculiar to a characterizing of the cancer cell.

TQOGp. The Genetics of the physics of cancer cell origin is concerned with the phylo- and ontogenetic derivation of the state of substance peculiar to and characterizing of the cancer cell.

TQOGa. The Genetics of the anatomy of cancer cell origin is concerned with the phylo- and ontogenetic derivation of the cell form and structure peculiar to and characterizing of the cancer cell.

TQOCc. The Chemistry of the chemistry of cancer cell origin is concerned with the substance and reactions of the substance productive of the chemical groups peculiar to and characterizing of the precancerous cell; and the substances and reactions which produce the cancer cell.

TQOCg. The Chemistry of the genetics of cancer cell origin is concerned with the substance and reactions of the substance through which the persistence of the chemical groups which characterize cancer cell production are maintained.

TQOCp. The Chemistry of the physics of cancer cell origin is concerned with the manner in which substance and reactions of substance determine the state of substance and changes in state of substance contributing to and characteristic of cancer cell production.

TQOCa. The Chemistry of the structure of cancer cell origin is concerned with the manner in which substance and reactions of substance produce the form and structure and changes in form and structure attendant upon cancer cell production.

TQOPp. The Physics of the physics of cancer cell origin is concerned with the changes in state of substance incident to the change from the precancerous to the cancer cell.

TQOPg. The Physics of the genetics of cancer cell origin is concerned with the state of substance and changes in state of substance attendant upon the phylo- and ontogenetic derivation of the cancer cell.

TQOPc. The Physics of the chemistry of cancer cell origin is concerned with the physical phenomena resulting from the chemical changes incident to the change from the precancerous to the cancer cell.

TQOPa. The Physics of the anatomy of cancer cell origin is concerned with the physical changes associated with the change in form and structure as the precancerous cell becomes a cancer cell.

TQOAa. The Anatomy of the anatomy of cancer cell origin is concerned with the changes in form and structure incident to the change from the precancerous to the cancer cell.

TQOAg. The Anatomy of the genetics of cancer cell origin is concerned with the form and structure and changes in form and structure attendant upon the phylo- and ontogenetic derivation of the cancer cell.

TQOAc. The Anatomy of the chemistry of cancer cell origin is concerned with the form and structure produced by substance and change in substance incident to the change from precancerous to cancer cell.

TQOAp. The Anatomy of the physics of cancer cell origin is concerned with the form and structure changes associated with the physical changes incident to the change from precancerous to cancer cell.

TQBGg. The Genetics of the genetics of cancer cell behavior is concerned with avenues and manner of maintenance of a strain of cells as cancer cells.

TQBGc. The Genetics of the chemistry of cancer cell behavior is concerned with the maintenance of transmissibility of the substance and reactions of substance within a strain of cancer cells.

TQBGp. The Genetics of the physics of cancer cell behavior is concerned with the maintenance of transmissibility of the characterizing physical phenomena of cancer cells.

TQBGa. The Genetics of the anatomy of cancer cell behavior is concerned with the maintenance of transmissibility of the form and structure of cancer cells.

TQBCc. The Chemistry of the chemistry of cancer cell behavior is concerned with substance and reactions of substance of cancer cells as such, and during the growth of the cancer cell and the cancer, and their reverberations.

TQBCg. The Chemistry of the genetics of cancer cell behavior is concerned with the reactions of cancer cell substance which derive from their inheritance as cancer cells; and which determine the maintenance of their transmissibility as cancer cells.

TQBCp. The Chemistry of the physics of cancer cell behavior is concerned with the rôle of substance and reactions of substance in the changes in state of substance of the cancer cell as such and during its growth.

TQBCa. The Chemistry of the anatomy of cancer cell behavior is concerned with the rôle of substance and reactions of substance in the production of cancer cell form and structure as such and during the changes incident to cancer and cancer cell growth.

TQBPp. The Physics of the physics of cancer cell behavior is concerned with the rôle of physical factors on the state of substance and changes in state of substance during cancer and cancer cell growth.

TQBPg. The Physics of the genetics of cancer cell behavior is concerned with rôle of the state of substance in the maintenance of transmissibility of cancer cells.

TQBPc. The Physics of the chemistry of cancer cell behavior is concerned with the rôle of state of substance and changes in state of substance on the substance and reactions of substance of the cancer cell as such and during its growth.

TQBPa. The Physics of the anatomy of cancer cell behavior is concerned with the rôle of state of substance and changes in state of substance in producing the form and structure characteristic of cancer cells as such and during their growth.

TQBAa. The Anatomy of the anatomy of cancer cell behavior is concerned with the structural elements going into the form and structure of the cancer cell and the cancer during their growth.

TQBAg. The Anatomy of the genetics of cancer cell behavior is concerned with the maintenance of transmissibility of cancer cell form and structure during cancer and cancer cell growth.

TQBAc. The Anatomy of the chemistry of cancer cell behavior is concerned with the form and structure produced by substance and change of substance attendant upon cancer and cancer cell growth.

TQBAp. The Anatomy of the physics of cancer cell behavior is concerned with changes in form and structure associated with changes in state of substance incident to cancer cell growth.

TQERGg. The Genetics of the genetics of cancer cell end result is concerned with the persistence of transmissibility of cancer cell characteristics in ex- and transplantation.

TQERGc. The Genetics of the chemistry of cancer cell end result is concerned with the persistence of the characterizing substance and reactions of substance in the mature cancer and its derivatives.

TQERGp. The Genetics of the physics of cancer cell end result is concerned with the persistence of the characterizing state of substance of cancer cells in the mature cancer and its derivatives.

TQERGa. The Genetics of the anatomy of cancer cell end result is concerned with the derivation and persistence of the characterizing basic form and structure of cancer cells in the mature cancer; its architecture; and its derivatives.

TQERCc. The Chemistry of the chemistry of cancer cell end result is concerned with substance and reactions of substance of the cells of the mature cancer, their derivatives, and their reverberations.

TQERCg. The Chemistry of the genetics of cancer cell end result is concerned with the substance and reactions of substance responsible for persistence of the characterizing attributes of the cancer strain of cells in the mature cancer and its derivatives.

TQERCp. The Chemistry of the physics of cancer cell end result is concerned with the rôle of substance and reactions of substance in the production of the state of substance in the mature cancer and its derivatives.

TQERCa. The Chemistry of the anatomy of the cancer cell end result is concerned with the relation of substance and reactions of substance of the mature cancer to the form and structure of the cancer cells and to its architecture.

TQERPp. The Physics of the physics of cancer cell end result is concerned with the influence of physical factors on the physical state of the mature cancer and cancer cells.

TQERPg. The Physics of the genetics of cancer cell end result is concerned with the rôle of state of substance in the persistence of the cancer cell in the mature cancer and its derivatives.

TQERPc. The Physics of the chemistry of cancer cell end result is concerned with the rôle of state of substance on the substance and reactions of substance of the mature cancer cells and cancer and its reverberations.

TQERPa. The Physics of the anatomy of cancer cell end result is concerned with the rôle of the state of substance in the cells of the mature cancer in producing the form and structure of the cancer cells and the cancer; its architecture; and its derivatives.

TQERAa. The Anatomy of the anatomy of cancer cell end result is concerned with the structural components and architecture of the mature cancer and the deformations produced thereby.

TQERAg. The Anatomy of the genetics of cancer cell end result is concerned with the persistence of characteristic cell form and structure in the mature cancer and its derivatives.

TQERAc. The Anatomy of the chemistry of cancer cell end result is concerned with the relation of the form and structure of the mature cancer and cancer cell to the substance comprising the same.

TQERAp. The Anatomy of the physics of cancer cell end result is concerned with the relation of cancer and cancer cell form and structure to the state of substance of the cells comprising the same.

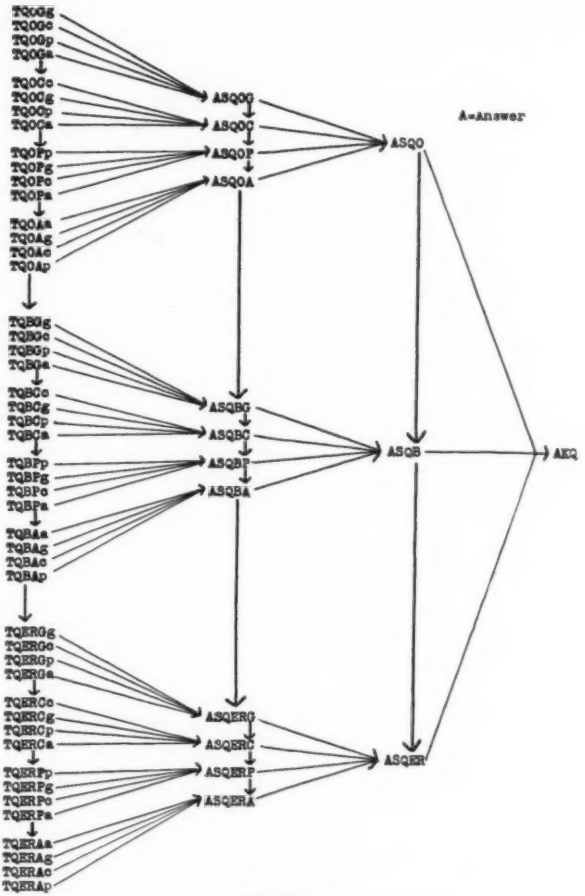

FIGURE 4

THE PATHS OF INTEGRATION FOR ANSWER TO THE KEY QUESTION: THE "*TAE*" DERIVATIVES

The *TAE* relations are visualized in Figure 4. In order to avoid confusion the lines showing the direct *O-ER* relations are omitted as are the lines showing the conditioning relations existing among the *AE* factors. These are easily derived from preceding diagrams.

The individual worker will undoubtedly wish to restate many of the expositions in his own terms in order to clarify his concepts

and understanding. Because of this, and because of the desire for brevity, I have in some instances refrained from pointing out all the associations inherent in the relations. The attempt has been made, however, to indicate all the salient points of contact in each exposition.

It is of course understood that the normal non-malignant cell is used as control in all the indicated procedures.

If the foregoing has demonstrated the possibility of integration and its usefulness and necessity, even in getting the answer to such an apparently simple question as the one posited here, the purpose of this presentation will have been accomplished.

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Growth, 1941, **5**, 171-173.

VARIATION AND CHANGE OF SIGN OF THE RELATIVE GROWTH RATIO FOR LARVAL SALAMANDER EYES TRANSPLANTED ON STARVING HOSTS

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(Received for publication, March 7, 1941.)

The relative growth of a part (y) with respect to that of the whole organism (x) may be expressed by the well-known formula $y = bx^{\alpha}$. It is often true, especially during ontogenesis, that α is not a constant and a complete understanding of the growth requires an analysis of the changes of the relative rates, $(dy/ydt)/(dx/xdt)$ of which α is the ratio (Richards, 1935).

Growth is defined herein as a measurable change in the size of the biological unit measured. Increasing size is positive growth and decreasing size is negative growth. Four general types may be anticipated: (*a*) the relative growth rate of the part and of the whole, both positive, $\alpha+$; (*b*) the relative growth of the part positive, that of the whole negative, $\alpha-$; (*c*) the relative growth of the part negative, that of the whole positive, $\alpha-'$; (*d*) the growth rates of both the part and the whole negative, $\alpha+'$. (To avoid confusion between classes, a prime (′) has been added to the sign of α in Classes *c* and *d*.) Within each type α may be less than, equal to, or greater than unity, depending on the numerical values of the relative rates of growth.

There are, at present, few sets of experimental data which permit analysis of the last three types. Twitty and DeLanney (1939) grafted salamander eyes from larvae of about the same size onto hosts which were not fed, and measured the changes in size of the diameters of the eyes and of the body lengths of the hosts. The younger Amblystoma tigrinum eyes gained in proportion to the *A.* punctatum host eyes, probably due to the greater growth potentiality of the younger tissue. Professor Twitty very kindly furnished me with a large graph of their Figure 1 from which the numerical data were scaled and analyzed.

The double logarithmic graph of the size of the eyes to that of the host body length Figure 1*A*, does not give straight lines, which indicates that the relative rates of growth and α are changing with time. The actual changes in the relative growth rates are shown in Fig-

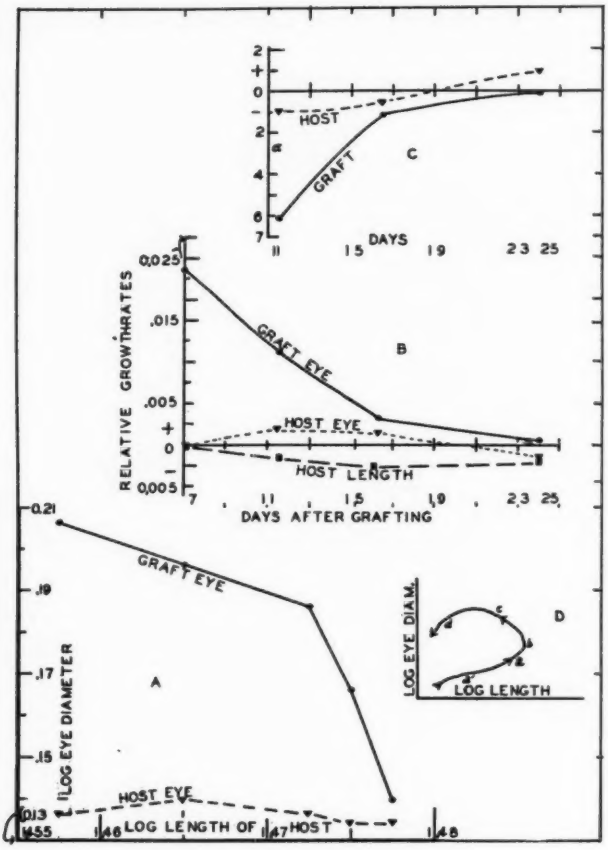

FIGURE 1

A and *D*. Relative changes in size.
B. Relative growth rates.
C. Variation of the relative growth ratio. (Cf., text.)

ure 1*B*. The relative growth of the host's body lengths is negative, first increasing and then decreasing at a gradually slowing rate. The host eyes grow positively until about the twenty-first day when their relative rate of growth becomes negative. The transplanted tigrinum eye grows positively at a decreasing relative rate of growth. The ratio for the relative rates of growth, α, changes from —6 to —0.2

for the transplanted tigrinum eye and from —1 to +0.9 for the host eyes, Figure 1*C*. The change of sign in the latter case is due to the change of sign of the relative growth rate of the host eye. The ultimate limitation of nutrition shifts the growth from Type *b* to Type *d*.

Even though based on very few data the changes of the relative growth ratio (α) are distinct and emphasize the need for broadening our conceptions and terminology for relative growth. Only in special cases of developmental growth is α a constant. The size of the host eye with respect to body length increases, (*a*) Figure 1*D*, until the period of starvation slows the rate (*c*); later the growth becomes negative. In fact, as Mr. A. J. Kavanagh pointed out when he read the manuscript, at (*b*) α increases to and passes through infinity. The more complete information gained from the actual relative rates of growth and the changes in α stressed earlier (Richards, 1935) is again apparent. Rather than establish or change terminology in small steps it may be preferable to depend on adequate and correct description until sufficient study of all kinds of growth permits an unambiguous terminology. Within limits the effects of starvation may be reversed and the analysis of the changes should add to our knowledge of the nature of growth.

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Growth, 1941, **5**, 175-181

THE INFLUENCE OF TISSUE FLUID ON HAIR GROWTH

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(Received for publication, April 7, 1941.)

Tissue fluid may be conducive to hair growth since increased vascularization and edema of the skin (Butcher, '40) induce activity in the quiescent hair follicles of the rat. This assumption leads one to suspect that there may be changes in the fluid content of the skin of normal rats and that the hair cycles in the rat—approximately 34 days long and about equally divided into a resting period and a growing stage—are partly dependent upon these changes.

Whether or not there are cyclic fluid changes in the skin of animals has not been carefully determined. Such an investigation is highly desirable since the subject of water balance is so widely studied, having been extensively reviewed by Adolph in 1933.

Besides investigating the fluid changes in the skins of normal animals in the present study, the fluid content of the skins of underfed animals has also been determined to learn if the retardation of hair growth upon underfeeding is partly due to the lack of fluid. In addition, the fluid conditions of the skin after adrenalectomy have been investigated to see if they are responsible for the acceleration of hair growth following the removal of the adrenals. Hartman ('32) has claimed that the water content of rats' skins actually increases after adrenalectomy, and it has been pointed out in a previous paper (Butcher, '39) that possibly the acceleration of hair growth after adrenalectomy is due to an increase of fluid in the skin.

All of the present investigations show that tissue fluid is conducive to hair growth, but that other factors are more necessary for hair growth. Merely increasing the fluid content of the skin will not induce hair growth. The tissue fluid content of the skins of normal rats changes very rapidly, and neither underfeeding nor adrenalectomy greatly alters the fluid content of the skin. These various determinations are of such interest and of such importance for other work that they warrant reporting.

A. THE FLUID CHANGES IN THE SKINS OF NORMAL RATS

It seemed desirable for comparative purposes first to determine

if there were changes in the fluid content of normal rat skin when the hair follicles were both active and resting.

To learn this—different aged rats were sacrificed. In order to eliminate the manner of killing as a causative factor of the amount of fluid, some animals were killed with ether while others were struck over the head. A large area of skin, 2 to 3 cm. wide and 4 cm. long, was then quickly removed from the dorsum and all adhering fat below the panniculus carnosus was dissected away. The skin was then placed in a porcelain crucible, weighed, and desiccated to a constant weight in an electric oven at a temperature of 110 degrees *C*. After desiccation the percentage of fluid loss was calculated.

Determinations were not begun until the rats were 10 days old since maximal activity is not reached in all follicles until about this age (Butcher, '34). The fluid content of the skin, including the hair, at the age of 10 days averaged 67.13 per cent (Table 1). At the age

TABLE 1

SHOWING PERCENTAGE OF FLUID IN SKIN WITH AND WITHOUT HAIR

Age in days	With hair		Without hair	
	No. of animals	Av. percentage of fluid content	No. of animals	Av. percentage of fluid content
10	3	67.13	3	69.35
15	5	64.18	5	67.49
20	4	57.26	3	61.30
25	6	56.28	3	61.14
28	5	61.98	3	68.55
31	2	67.02	4	72.35
35	8	67.32	2	73.64

of 15 days the fluid content had decreased to 64.18 per cent. By the 20th day, the fluid content (57.26 per cent) had almost reached its minimal level.

Very soon after the 20th day of life, all hair follicles terminate growth. If the rats are undersized on account of undernutrition, the hair continues to grow over a longer period or until it reaches its definitive length. In this instance, the minimal fluid level is not reached until the 22nd or 23rd day.

Between the age of 10 and 20 days the quantity of hair increases externally and as the hair contains little water, the percentage of fluid in the skin including hair accordingly decreases. In order, therefore, to determine more accurately the fluid condition of the skins of rats 10 to 20 days, skin without hair was desiccated (Table 1). After the 20th day of life, the quantity of hair remains practically the same

until the next cyclic growth, so that changes in fluid content can be accurately determined by desiccating the skin with the hair. Determinations of older animals without the hair have been made however (Figure 1).

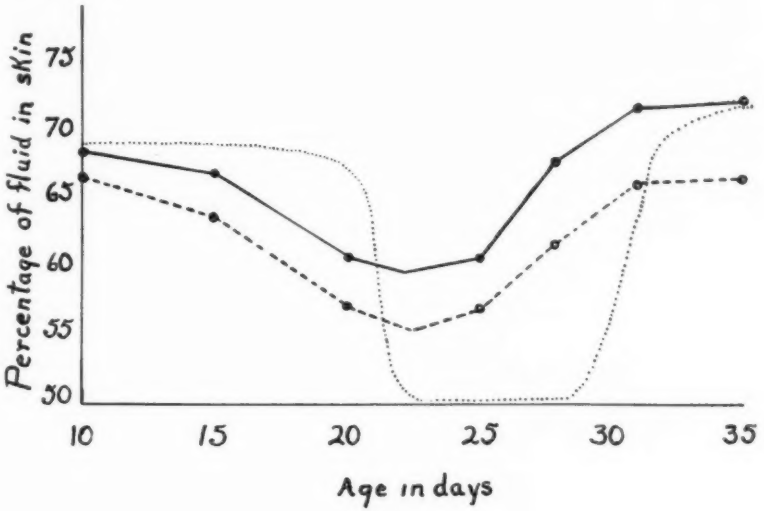

FIGURE 1

CHART SHOWING THE FLUID CHANGES IN THE SKIN

——— line, skin without hair; – – – – line, skin with hair; . . . . line, shows activity of hair follicles—active until 20th day—inactive until 30th day.

Soon after the age of 23 days (Figure 1) the fluid content of the skin begins to increase rapidly and by the 30th day of life it has almost reached the level occurring at the age of 36 days when the second growth of hair appears externally. Activity in the buds for the second growth of hair seldom begins before the 30th day of life. The fluid content, therefore, increases considerably before growth begins (Figure 1).

A section of the skin of a 21-day-old rat—fluid content with hair, 57.38 per cent—is shown in the left photograph (Figure 2). Practically all hair follicles are inactive and are rising to the resting level. The skin from a 28-day-old litter mate is shown on the right. The fluid content in the latter has increased to 62.29 per cent although there is less cellular activity in the hair buds than in the 21-day-old

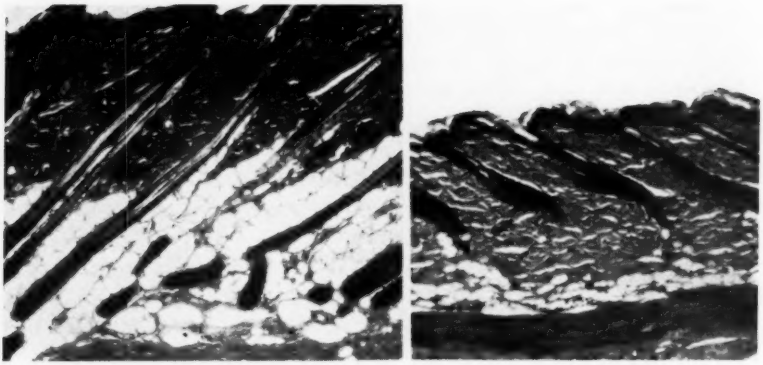

FIGURE 2

At left, rat 21 days old, fluid content of skin with hair—57.38 per cent.
At right, litter mate rat 28 days old, fluid content of skin—62.29 per cent.

rat. Growth in the hair bulb does, therefore, not account for the increase of fluid in the skin.

B. The Fluid Content of the Skins of Underfed Animals

The fluid content of the skins of underfed animals[1] was determined when they were usually 37 days old in a similar manner to the method used in determining the fluid content of normal animals. Skins of 19 underfed animals (hair removed) were desiccated and the fluid content averaged 73.88 per cent which is practically the same as the average fluid content of the skins of normal animals producing hair (73.64 per cent). The fluid content of the skin was, therefore, not entirely responsible for the growth of hair, or it would have grown in these underfed rats.

C. Fluid Changes in the Skin Following Adrenalectomy

Hartman's Figure 4 ('32) indicates that the water content of skins of rats increases about 3.5 per cent after adrenalectomy. Since the fluid content of the skin changes 5-7 per cent in the normal animal within an interval of 3-5 days, it is entirely possible that this percentage may be of little significance unless very similar litter mates are used. Because the fluid content is at its minimal level for such

[1]Weaned and isolated at the age of 22 days, and thereafter fed only sufficient finely ground Purina dog chow, usually two grams in the morning and two in the evening, to prevent a loss in body weight.

a short interval in well fed rats it seemed advisable to use underfed litter mates whose skin remains approximately the same for a longer period, and the effects of adrenalectomy can more accurately be determined. The fluid changes in adrenalectomized well fed animals have, however, been studied.

To determine if there were a fluid increase in the skins following adrenalectomy, many underfed rats were adrenalectomized via the ventral approach usually when 36 days old. At various intervals after removal of the adrenals, operated and unoperated litter mates were injected intraperitoneally with different amounts and different concentrations of either trypan blue, toluidin blue, methylene blue, vital red, eosin, gentian violet, or congo red. The intensity of color and the interval necessary for the stain to appear in the skin after injection served to indicate the extent of capillary permeability and increased fluid content. Another method consisted of comparing the fluid content of skins from the backs of operated and unoperated animals by desiccating at various intervals after adrenalectomy.

It was not possible with the dyes just listed and which have been employed by others (Landis, '27; Rigdon, '39) in studying capillary permeability to show that there was any greater passage of fluid through the capillary wall in the adrenalectomized rats than in the unoperated litter mates. The dorsums of all from which the hair had been previously removed with sodium sulphide colored equally in intensity and equally as rapid after injection of the various solutions.

Determination of the fluid content of the skins of 19 adrenalectomized animals by desiccation showed that the fluid content of their skin averaged only 1.4 per cent (standard deviation, 1.76) more than 19 unoperated litter mates. The interval between adrenalectomy and sacrificing the animal (ranging from 40 hours to 6 days in these experiments) had no effect on the percentage of fluid in the skin. Skin with and without hair was desiccated and sections of the skin were examined to see if adrenalectomy had induced hair growth. The fluid content of the skin of 17 adrenalectomized, well fed animals averaged only 1.13 per cent more than the fluid content of 18 well fed, litter mates.

Since the skins of underfed animals have as much fluid as the skins of normal animals which are producing hair, and adrenalectomy induces such a small increase in fluid content, it seems that hair growth upon adrenalectomy is due to some other factor than the fluid content of the skin. It is true, however, that fluid content is conducive

to hair growth because when underfed animals whose fluid content is high are adrenalectomized, they regularly produce hair externally in nine days. If well fed 22-day-old rats whose skin is normally low in fluid content are adrenalectomized, hair seldom grows externally in less than 10 or 12 days.

D. Discussion

The present investigations show that the fluid content of the skin of the normal animal decreases before the quiescent phase of the hair cycle begins, and that there is a fluid increase previous to the time of hair growth. These changes lead one to think that the fluid content is partly responsible for the length of the active and inactive periods of the hair cycle. However, when the skins of the underfed rats, whose hair follicles are inactive, are examined, it is found that their fluid content is equal to that of normal animals whose hair is growing. This finding indicates, therefore, that the fluid content is not the only factor necessary for hair growth.

There is the possibility that the variations in the fluid content of normal animals may be due to changes in the fatty content of the skin. For instance, the fatty content of the skin may decrease in the normal animal 10 per cent between the 10th and 20th days, remain stationary after weaning for a few days and then increase as the animal begins to grow and mature sexually. Histological sections, however, do not show that such changes in the fat content of the skin occur. It has also been pointed out elsewhere (Butcher, '34) that hair growth occurs often when there is little or no fat present. The best evidence that can be presented that fat changes do not account for the fluid changes comes from the removal of the adrenals in underfed animals. These animals retain almost the same weight after adrenalectomy. There has been no increase in fat as far as can be determined histologically when the hair starts to grow.

Upon adrenalectomy of underfed rats the fluid in the skin increases very little, which shows that some other change or changes are responsible for the immediate growth which follows the removal of the adrenals. Grollman ('36) also claims that the tissues of animals dying of adrenal insufficiency never show an abnormal hydration. Likewise, Silvette and Britton ('33) say that the water content of the skin of cats is not affected by adrenalectomy. The fluid content is important, however, because adrenalectomized animals with high fluid skin content (underfed) produce hair sooner than adrenalectomized animals with low fluid content (22-day well fed rats).

E. Conclusions

1. The fluid content of the skin of normal animals decreases before the quiescent phase of the hair cycle begins and increases prior to the time of hair growth.

2. Underfeeding does not affect the tissue fluid content of the skin, and the retardation of hair growth upon underfeeding is due to other factors.

3. Since there is little increase of fluid after adrenalectomy, the removal of adrenals does not accelerate hair growth via tissue fluid.

4. Tissue fluid is conducive to hair growth, but other factors are more necessary for hair growth.

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Growth, 1941, **5**, 183-195.

THE GROWTH OF TRANSPLANTED MINUTE AND WILD TYPE OPTIC DISKS IN DROSOPHILA MELANOGASTER

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(Received for publication, April 10, 1941.)

A. INTRODUCTION

In connection with problems of tissue growth in development and pathology, it is of interest to know whether host tissue can affect the rate of growth of transplanted tissue when the donor differs in growth rate from the host. A direct test of such an effect can be made by means of transplantation in Drosophila melanogaster, with the advantage that tissues can be used which have been analyzed genetically. In the investigation here described the optic disk was selected as the experimental tissue because facet counts of the transplanted eye after the completion of development afford a quantitative measure of the growth by cell division which has taken place. An attempt has been made to ascertain whether growth of the transplanted disk is altered in a host of different growth rate, as compared with growth in a host of the same growth rate.

The tissues used were those of wild-type larvae and of larvae heterozygous for Minute(3)w. M(3)w, a dominant factor at locus 79.9 in the third chromosome, is lethal when homozygous; the heterozygous adult is characterized by short, fine bristles and smaller body size than the wild type. Although the duration of the embryonic period to hatching from the egg is the same in the wild type and heterozygous Minute, the larval period of the Minute is prolonged to the following extent at 25°C (Brehme, 1939a): Duration of the larval period in hours: wild-type ♂ 87.2 ± 3.0; wild-type ♀ 86.6 ± 3.0; Minute ♂ 128.5 ± 3.0; Minute ♀ 129.5 ± 3.0. (The error of ± 3 hours is based upon an interval of 6 hours between observations). Each larval instar is lengthened; the first moult occurs one hour later than that of the wild type; the second moult occurs three hours later than that of the controls; while the Minute males form puparia 41 hours later, the

[1]The work was begun at Stanford University during the tenure of a national fellowship of the American Association of University Women. The author is grateful to the faculty of the School of Biological Sciences, Stanford University, for the facilities of the laboratory, and particularly to Professor G. W. Beadle for advice and for instruction in the technique of transplantation.

females 43 hours later than the wild type. Whether or not there is a significant prolongation of the pupal period has not been conclusively shown.

Both larval and imaginal tissues of M(3)w are characterized by a difference in growth rate from the wild type. The growth curves of larval length and width respectively, plotted against time, show a general similarity in shape, although the Minute curves are spread over a greater number of hours (Brehme, in press). One hour after hatching, however, the width of the Minute larva is significantly less than that of the wild type, although it does not differ in length; at the time of puparium formation of the wild type, the Minute is smaller in both length and width; and at puparium formation of the Minute, it is still significantly less in width, but has slightly surpassed the wild type in length. Relative growth curves of Minute and wild type larvae are almost identical, showing no significant difference in k or b in Huxley's formula $y = bx^k$. In general, therefore, the type of growth of the Minute and wild-type larvae is the same, but the Minutes grow more slowly. An effect on growth of imaginal tissue has been observed by Parshley (1941), who found that at 48 and 72 hours after hatching, the optic disks of the wild-type larvae are about twice as large as those of Minute(3)Florida, as shown by planimeter measurements of the area of flattened disks; at 122 hours, the M(3)Fla disks have attained approximately the same size as 96-hour wild-type disks. The rate of growth of the optic disk of M(3)Fla is therefore slower than that of the wild type. Such an effect has been observed, although no measurements were made, in histological preparations of 48-hour old M(3)w larvae (Brehme, 1939a). Similarity between the growth of the optic disks of M(3)w and its allele, M(3)Fla, is to be expected, since these two Minute factors have been found to have almost identical effects on developmental rate, the larval growth curve and adult body size (Brehme, 1939a and in press).

The effect of the host upon growth of transplanted optic disks has been studied by Bodenstein (1939, 1940), whose approach to the problem differs in one very important respect from that of the present investigation. Bodenstein, in all his experiments, used donors and hosts of the same genotype, the development of whose optic disks follow the same growth curve; he studied the effect of tissue growing at a rate characteristic of one part of its growth curve upon tissue at another stage in the same growth curve. The present investigation, on the other hand, deals with tissues of different growth curves.

B. Material and Methods

The stock used was a strain of M(3)w/ca on a background of Florida wild type, made isogenic by the use of marked chromosomes carrying crossover suppressors and maintained by crosses of Minute males by claret females. The Florida stock was originally obtained from Columbia University. The Malpighian tubes of the homozygous claret larva are colorless; the heterozygous Minute larvae could thus be recognized by the wild-type tube color, bright yellow. As the eye color mutation claret has no known effect on developmental rate or on size or structure of the eyes or other organs, the claret larvae will be referred to as wild type in the following account.

All cultures were maintained at 25°C. Egg collections were made on aluminum trays from mass matings in wide-mouthed mason jars (Clancy, 1939). Since it has been shown that there is no difference in the length of the developmental period to hatching of M(3)w and wild type (Brehme, 1939a), and since there is great variability in the interval between oviposition and hatching, due to delayed oviposition, the experimental larvae were timed in hours after hatching from the egg. Larvae were collected from the egg trays at 4-hour intervals; their age was thus known within ± 2 hours. They were cultured in finger bowls on a cornmeal-agar-molasses medium, seeded with a thick suspension of live yeast.

The optic disks of third instar larvae were transplanted by the method described by Beadle and Ephrussi (1936). No attempt was made to dissociate the optic from the antennal disk, although they often separated in the process of dissection from the donor. Where young wild-type larvae were donors, care was taken to use only those which had undergone the second moult, as optic disks of second instar larvae do not develop normally in third instar hosts. As the sexes differ in adult facet number, only females were used as experimental material. After the operation, the host larvae were placed in shell vials on slants of the food medium which had been autoclaved and subsequently seeded with live yeast suspension.

After eclosion of the hosts, the transplanted eyes were dissected out of the host abdomen, macerated in KOH, stained with acid fuchsin, and mounted in balsam (Bodenstein, 1939). They were then projected onto a screen with a photomicrograph projector and the facets were counted.

Preliminary reports of the results have been made in abstract (Brehme, 1939b and 1940).

C. Results

1. *Adult Facet Number*

In interpreting the results of the transplantation experiments, it is necessary to know whether a difference exists between the adult facet number of Minute(3)w and wild type. Accordingly, stained preparations were made of the right eyes of adult females from a single culture reared under the conditions used for the transplantation material. The facets were not counted directly, but the facet number calculated in the following manner. The slides were placed in a photomicrograph projector, and the area of the projected cornea was measured with a Keuffel and Esser planimeter. The facets within a square 0.0144 mm^2 in area at the center of the eye were counted; from this count the total number of facets in each eye was calculated. This method is legitimate in view of the fact that the facets of the adult eye are regularly arranged and do not differ in size in different parts of the eye. The results (Table 1) show that wild-type and Minute(3)w

TABLE 1

Facet Number and Facet Area in Adult Eyes of Wild Type and M(3)w

	$M \pm \sigma_m$	$\sigma \pm \sigma_\sigma$	Number
Total facet number			
+ ♀♀	726.4 ± 16.6	76.0 ± 11.7	25
M(3)w ♀♀	730.8 ± 22.7	106.5 ± 16.1	25
	Difference = 4.4 ± 28.1		
Facet area (number of facets in 0.0144 mm^2)			
+ ♀♀	67.2 ± 0.8	3.6 ± 0.6	25
M(3)w ♀♀	76.1 ± 2.4	11.0 ± 1.7	25
	Difference = 8.9 ± 2.5		

female eyes do not differ significantly in facet number. The actual figures, 726.4 facets for the wild type, 730.8 for M(3)w, are in close agreement with those obtained for wild type and M(3)Fla by Parshley (1941), who counted the facets of the entire eye and found 731.2 facets for the wild type female, 741.8 for the Minute. Apparently the Minute disks grow more slowly than the wild type but continue growth longer, during the interval of about 42 hours between puparium formation of the wild type and of the Minute, and ultimately form as many facets as the wild type.

The number of facets within an area of 0.0144 mm^2 was found to be significantly larger in M(3)w females than in wild-type females (Table 1); the Minute facets are therefore smaller. This is not in

agreement with the results obtained for M(3)Fla by Parshley, who superimposed camera lucida drawings of Minute and wild-type disks and found no difference in facet size. It is possible that M(3)w and M(3)Fla are unlike in this respect, but, since the difference shown in Table 1 is not great, it is possible also that such a difference would not be detectable by Parshley's method. A similarly reduced cell area has been found in the wing cells of M(3)w (Brehme, in press).

The Minute(3)w eye, with facets which are equal in number to those of the wild type but smaller in area, is therefore a smaller eye than that of the wild type. This is expected, since M(3)w has been found slightly smaller in tibia length and in wing area than the wild type (Brehme, 1939a and in press).

Great variability in facet number and facet area characterized both Minute and wild type eyes in the sample described in Table 1. However, measurements of wing area and wing cell area, made from the identical flies used in the eye measurements, show very low variability. No explanation suggests itself for this disproportionate variability of the two organs.

It has proved impossible to obtain accurate measurements of facet area in the transplanted eye, since the facets are often irregularly arranged or partially fused, and since they appear to differ somewhat in size in different regions of the eye (Figure 1*a*, for example), possibly as a result of the disturbing effect of the operation upon differentiation. The following analysis is therefore confined to growth by increase in cell number as expressed by the number of facets.

2. *Donors and Hosts of the Same Age*

Under the conditions of the experiments reported here, the wild-type larvae formed puparia at an average of 100 hours from hatching, the Minutes at 144 hours. Reciprocal transplants were made of Minute and wild-type optic disks into hosts of the same age as the donors, 76 hours (middle of the third instar). At this time, the Minute hosts had 68 hours to develop before puparium formation, the wild-type hosts, 24 hours. The data presented in Table 2 show that transplants of Minute disks form significantly fewer facets (295.1) in wild-type hosts which form puparia in 24 hours than in the Minute controls (481.7 facets) which, like the donors, do not form puparia for 68 hours. That the difference in facet number between the two sets of transplants is significant is indicated both by the standard error of the difference (186.6 ± 33.6) and by the t test

TABLE 2

FACET NUMBERS OF OPTIC DISK TRANSPLANTS AT ECLOSION

Transplant	Age at operation ± 2 hours donor	host	Hours before puparium formation of host[1]	Facet number $M \pm \sigma_m$	$\sigma \pm \sigma_\sigma$	Range	Number
M(3)w in +	76	76	24	295.1 ± 23.9	82.7 ± 16.9	132 - 474	12
M(3)w in M(3)w	76	76	68	481.7 ± 23.6	78.5 ± 16.7	365 - 582	11
				Difference = 186.6 ± 33.6			
				P much less than .01			
+ in +	76	76	24	489.1 ± 28.8	99.6 ± 20.4	345 - 644	12
+ in M(3)w	76	76	68	599.5 ± 23.1	86.4 ± 16.3	399 - 711	14
				Difference = 110.4 ± 36.9			
				P less than .01			
M(3)w in M(3)w	108	108	36	601.6 ± 27.1	101.2 ± 19.1	477 - 761	14
M(3)w in +	108	64	36	646.4 ± 21.4	64.1 ± 15.1	556 - 740	9
				Difference = 44.8 ± 34.5			
				P between .3 and .2			
+ in +	64	64	36	605.5 ± 25.3	83.6 ± 17.8	450 - 759	11
+ in M(3)w	64	108	36	562.4 ± 10.6	35.1 ± 7.5	505 - 619	11
				Difference = 43.1 ± 27.4			
				P between .2 and .1			
[2]M(3)w in M(3)w	108	108	36	601.6 ± 27.1	101.2 ± 19.1	477 - 761	14
[2]+ in M(3)w	64	108	36	562.4 ± 10.6	35.1 ± 7.5	505 - 619	11
				Difference = 39.2 ± 29.1			
				P between .2 and .1			

[1]Calculated from developmental rate of unoperated animals.
[2]Data repeated for comparison.

of Fisher, where the probability that such a difference is due to chance is much less than .01. Wild-type disks form more facets (599.5) in Minute hosts which take 68 hours before puparium formation than in wild-type hosts (489.1 facets) which form puparia in 24 hours after transplantation, as the donors would. The difference is again significant, judging both by the standard error (difference = 110.4 ± 36.9) and the *t* test (*P* less than .01).

That the structure and arrangement of the facets is not grossly abnormal in the transplanted eyes is shown by photomicrographs (Figure 1). Irregularities in facet boundaries and facet and bristle arrangement were found in all groups of transplants, and were no more frequent when donors and host differed in age or genotype than when they were similar in these respects. Following the operation, no delay was noted in puparium formation of the hosts beyond the time characteristic of their genotypic growth rate.

It may be concluded from these data that the length of the interval

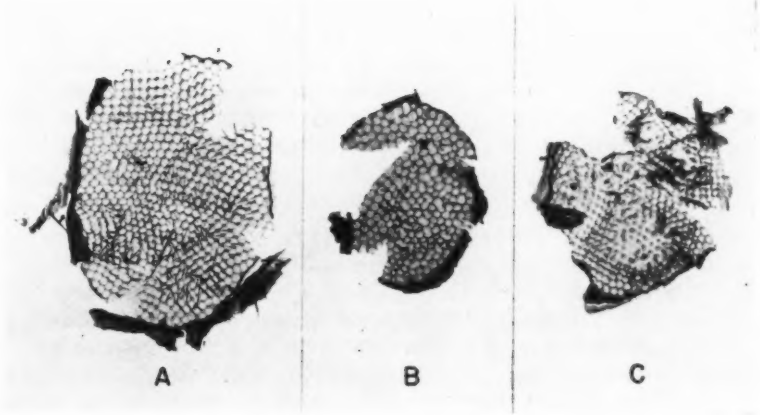

FIGURE 1

TRANSPLANTED OPTIC DISKS, WITH DONOR AND HOST OF THE SAME AGE, 76 HOURS (*a*) + in +; (*b*) M(3)w in +; (*c*) M(3)w in M(3)w. Photomicrographs × 112.

between transplantation and puparium formation of the host is a major factor in determining the facet number attained by the transplant. Apparently the wild-type and Minute disks in transplant continue growing at the rate characteristic of the donor (more slowly in the case of the Minute than the wild type) but stop increasing in number of facet rudiments at pupation of the host, regardless of the amount of growth which has gone on in the disk up to that time.

It is also evident that increase in facet number continues during the late third instar, as indicated by the data from wild-type disks in wild-type and in Minute hosts. Here a prolongation of the interval before pupation beyond that normally available to the disk results in the formation of more facets during the extra 44 hours. This finding is not in agreement with that of Enzmann and Haskins (1938), who are of the opinion that the maximum number of elements in the eye has virtually been reached at 70 hours after hatching. The possibility was suggested that in the 76-hour transplants of wild-type into Minute, cell divisions leading to facet formation are no longer occurring, but that tissue which would normally form parts of the eye other than facets is being converted into facet material. In order to ascertain whether cell divisions are actually taking place in the optic disk during the late third instar, acetocarmine smears were made of disks

of wild-type and Minute larvae which had crawled out of the food and were about to form puparia. Care was taken to dissect off the antennal disk, so that only optic disk tissue was present. In smears of both genotypes, numerous mitoses were found. Smears of disks from white puparia, within one hour of puparium formation, showed only occasional mitoses. It was thus demonstrated that cell division is still taking place in the late third instar larva, and is ended at about the time of puparium formation. Whether the observed mitoses are occurring in facet forming tissue or in some other tissue of the eye cannot be determined by this method, but taken in connection with the transplantation data in Table 2, it is evident that some at least of the cell divisions are taking place in facet forming material. It would therefore appear that the conclusion of Enzmann and Haskins is not correct.

The evidence from these transplants leads further to the conclusion that the formation of facet material in the optic disk is brought to a close by some effect of pupation, and not as a result of a growth cycle inherent in the disk. That this is the case is indicated by the experiments with M(3)w transplants in + hosts, where pupation occurs 44 hours earlier and fewer facets are formed than in the controls, M(3)w in M(3)w; here the growth cycle of the Minute disk, which would in situ require 44 hours more for completion, is interrupted by pupation of the host, although the number of facet rudiments which could be formed by the disk (as evidenced by the controls, M(3)w in M(3)w) is not attained. It is indicated also by the transplants of + in M(3)w, where the hosts pupated 44 hours later and more facets were formed than in the + in + group; although the normal growth cycle of the wild-type disks is probably completed before the host forms its puparium, the transplanted disks have continued to form facet rudiments during the extra 44 hours afforded them by the Minute hosts. Bodenstein (1939) arrived at the same conclusion, basing it upon reciprocal optic disk transplants of young and old Bar larvae.

Variability in facet number of the transplanted eyes was very great in all experimental groups. Three factors suggest themselves in explanation of this phenomenon. (*a*) The facet number of the unoperated adult of this stock is highly variable, as shown by the data of Table 1. (*b*) Donor and host larvae were collected over a period of four hours, allowing a maximum variation of four hours in the age of each group; the time required for the operation introduces a fur-

ther variation of about one hour. This variation in age, affecting the tissues at a time of rapid growth in the third instar, may be the cause of considerable variability in the facet number of the transplanted eyes. (*c*) Unoperated larvae of the same age normally form puparia over a period of several hours; the shock of the operation and the time required for the hosts to recover from the anaesthetic and resume feeding would be expected to increase the range of time of puparium formation. The hour of puparium formation, given in Table 2, was calculated from the time known to be correct for unoperated animals, and agreement of the experimental material with this calculation was not checked by observations at short intervals. Such variation in the number of facets formed by transplanted disks has been observed also by Bodenstein (1939), although some of the variability noted in his material is probably due to the fact that he did not classify donor or host larvae for sex, which is known to affect the facet number.

3. *Donors and Hosts with the Same Interval before Pupation*

Since the interval between transplantation and pupation has been demonstrated to be an important factor in determining the number of facets formed by the transplant, a test for an effect of host tissue upon the growth rate of the donor tissue can only be made with this interval eliminated as a variable. Accordingly, larvae were used as donors and hosts at an age equally distant, 36 hours, from the time of puparium formation of each genotype; at this time, the wild-type larvae were 64 hours, the Minutes 108 hours from hatching. Following the operation, the hosts were observed to form puparia within eight hours of each other; observations were not made at shorter intervals than eight hours.

The results (Table 2) show that growth of the transplanted disks was not changed in any group of transplants into hosts of different growth rate. M(3)w disks in M(3)w hosts and in + hosts attain mean facet numbers which are not significantly different by the criteria of standard error or the *t* test of Fisher (difference = 44.8 ± 34.5, with *P* between .3 and .2). Similarly, disks of + donors in + and in M(3)w hosts do not differ significantly in the number of facets formed (difference = 43.1 ± 27.4, with *P* between .2 and .1). The mean facet numbers formed by transplants of M(3)w and + disks in M(3)w hosts have further been compared; the difference between the means (difference = 39.2 ± 29.1, with *P* between .2 and .1) indicates that the Minute and wild-type disks grow equally well in the Minute host.

As in the transplants with donors and hosts of the same age, no gross abnormalities of facet formation were observed (Figure 2).

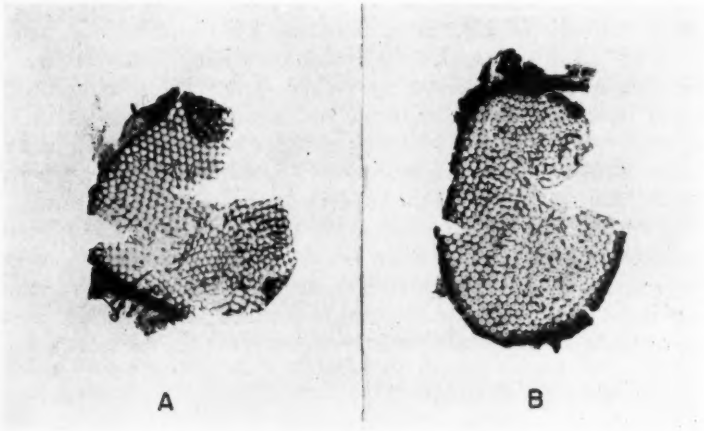

FIGURE 2

TRANSPLANTED OPTIC DISKS WITH 36 HOURS OF HOST LARVAL LIFE FOLLOWING THE OPERATION

(*a*) 64-hour + disk transplanted into 108-hour M(3)w host; (*b*) M(3)w in M(3)w, donor and host both 108 hours old. Photomicrographs × 112.

Again, variability in final facet number was very great. It may be noted that transplants of M(3)w in M(3)w with 68 hours before pupation (76-hour tissues) formed fewer facets (481.7 ± 23.6) than in the later experiment, M(3)w in M(3)w 36 hours before pupation, (601.6 ± 27.1). It is possible that this irregular result is attributable to the greater inhibitory effect of an operative shock which occurred at an earlier age in the 76-hour larvae.

It may be concluded that no effect upon the growth of transplanted Minute(3)w or wild-type optic disks by host tissue of different growth rate has been demonstrated.

D. DISCUSSION

As pointed out above, the problem of the effect of the host upon growth of transplanted tissue has been differently approached by Bodenstein, who used tissues of the same genotypic growth rate at different stages in their growth curves. In one series of experiments with a Bar stock of Drosophila melanogaster (Bodenstein, 1939)

young third instar optic disks were transplanted into older hosts, and disks of late third instar donors into younger hosts of the same instar. It was found that disks remaining a longer time in the host develop more facets than disks having a shorter time at their disposal for development. This conclusion is in agreement with the results obtained by the different experimental means reported in the present paper. Bodenstein further altered the time available for growth of the transplant by complete starvation of the host after the 70-hour change, which accelerates puparium formation, and by partial starvation begun before the 70-hour change, which prolongs the larval period. In the former case, the transplanted eyes showed a slight decrease in facet number; in the latter case, a great increase in facet number was observed in some cases.

Although these results of Bodenstein are in general agreement with those reported above, the investigation of Steinberg (1941a and b) of the development of the Bar eye indicates that Bar does not provide good material for the problem under consideration. Steinberg has found that the Bar disk is smaller than the wild-type disk at 36 hours from hatching, but has a similar growth curve. However, facet rudiments, which appear in the wild-type disk in the larval stage, are not found in the Bar disk until after pupation. Furthermore, transplantation experiments of Beadle and Ephrussi (1936) have shown that the Bar eye disk forms less v^+ substance than the wild type, an additional difference between the physiological processes occurring in the two types of disk. Apparently the Bar disk differs very greatly from the wild type in its development and experiments with Bar tissue are difficult to interpret until more complete information is at hand.

It is further suggested that complete and partial starvation are not simple means of shortening and prolonging development, but very possibly have a complex effect on metabolism. That this is probable is indicated by the production of v^+ substance by *v bw* larvae fed at a low food level during a sensitive period in the larval stage, although fully fed *v bw* larvae do not produce the substance (Beadle, Tatum and Clancy, 1938).

It has been found by Ephrussi, Khouvine and Chevais (1938) that Bar larvae fed upon Calliphora extracts rich in v^+ substance form more facets than Bar larvae not experimentally fed; there thus appears to be a relation between the v^+ substance and a stimulus to facet formation. This is an additional reason why starvation, especially of Bar larvae, would seem to introduce effects on development which cannot at this time be explained.

E. Summary

An attempt has been made to determine whether host tissue can affect the rate of growth of transplanted tissue when the donor differs in growth rate from the host. Tissue of Minute(3)w larvae was used as that growing at the slower rate, wild-type tissue as that growing more rapidly. Optic disks of Minute(3)w and of wild-type female larvae were transplanted into host larvae of the same and of the alternative genotype. It was found that the length of the interval between transplantation and puparium formation of the host is a major factor in determining the number of facets formed by the transplanted eye. Increase in facet number of the transplant was not found to be affected by a host of the different growth rate when the interval between the operation and puparium formation was the same in both donor and host. The evidence from transplantation experiments was supported by observations of acetocarmine smears of larval and pupal optic disks in indicating that cell division leading to facet formation is still taking place in the late third instar, but is virtually ended at puparium formation. It was further found that the facet number of adult Minute(3)w and of wild-type females is not significantly different, although the area of the individual facet is smaller in Minute than in wild-type eyes.

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PROTOZOA NEED CARBON DIOXIDE FOR GROWTH*

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On page 113 of this volume, the statement of Valley and Rettger that all bacteria need carbon dioxide for growth had been shown to apply to young as well as old cells. Although most bacteria produce CO_2 by respiration or fermentation, it is possible by the right technique to remove it as rapidly as it is produced, and under these circumstances, bacteria cannot multiply.

This study has now been extended to one alga and two protozoa, and it was possible to demonstrate that both types of organisms must have CO_2 for multiplication. With algae, as chlorophyll plants, this may not seem surprising, but the cultures were kept dark, in a medium containing glucose and citric acid. In this medium, the species tested, an unidentified *Chlorella* (No. 11 of the Cornell Laboratory of Plant Physiology) grew well in complete darkness to large numbers, producing chlorophyll. The alga obtained the growth energy, and perhaps the building material, from the organic matter; it lived essentially like a bacterium.

The method of testing the CO_2 requirement was the same as with bacteria. The alga was grown in pure culture in tiny drops of medium hanging from a sterilized cover-glass which was sealed airtight to a microculture slide by a ring of vaseline. At the bottom of the cavity was a small drop of 2.5 per cent KOH for the culture without CO_2, or of 2.5 per cent KCl for the control with a normal CO_2 content of the atmosphere.

Table 1 shows a typical experiment. With a heavy inoculum—100

TABLE 1

MULTIPLICATION OF CHLORELLA WITH AND WITHOUT CO_2

Numbers indicate cells per droplet.

	CO_2	No CO_2	CO_2	No CO_2	CO_2	No CO_2
Start	100	100	30	40	14	4
2 days	360	350	69	22	9	4
6 days	>800	>800	490	120	63	4
11 days	moldy	lost	dried	250	149	3

cells of this fairly large Chlorella in 2 mg. of medium is quite a large initial population—the CO_2 was produced more rapidly than it could be removed, and there is no difference between test and control. With the smaller inoculum of 30 to 40 cells per drop, CO_2 removal made an impression by causing a very definite delay, and slower multiplication. Very small numbers of cells could not get started at all, and the cells never multiplied in 11 days. This checks well with the behavior of bacteria.

Two pure cultures of flagellates were tested in the same way, *Astasia longa* and *Polytomella caeca,* which I owe to the kindness of Professor R. P. Hall of Columbia University. These animals thrive well in a solution of 1.00 per cent tryptone plus 0.05 per cent KH_2PO_4. They were tested in hanging droplets, but the KOH and KCl solutions had to be reduced to 1.00 per cent because of the weak osmotic pressure of the medium.

TABLE 2

MULTIPLICATION OF PROTOZOA WITH AND WITHOUT CO_2

Numbers indicate motile cells per droplet.

		CO_2	No CO_2	CO_2	No CO_2
Polytomella	Start	250	163	14	11
	1 day	Very many	165	66	19
	2 days	620	163	101	16
	3 days	Very many	46	—	3
	6 days	175	0	98	0
Polytomella	Start	225	153	11	11
	1 day	300	164	38	19
	2 days	285	71	48	16
	3 days	44	5	27	3
	6 days	Bacteria	0	27	0
Astasia	Start	13	29	5	2
	1 day	27	32	7	2
	2 days	33	27	15	2
	3 days	54	33	28	2
	6 days	85	33	140	2
Astasia	Start	30	20	5	5
	3 days	60	25	10	7
	6 days	100	21	65	5
	11 days	270	10	183	4*

*Dead.

Typical results in Table 2 show that it is quite possible to remove CO_2 as rapidly as it is produced. Since both organisms are motile, at times even rapidly motile, the counts of the individuals show considerable fluctuation. Yet, there is no doubt that all cultures with

CO_2 grew, and those without it did not. Only motile cells were counted, because it seemed impossible to ascertain which of the non-motile cells were still alive. These protozoa seem to respond more promptly to CO_2 removal than algae or bacteria, be it that their demand is greater, or that their rate of respiration per mg. body weight is smaller. *Polytomella* is quite sensitive and dies easily. *Astasia* is more resistant.

The rôle of the CO_2 is not known. Valley and Rettger observed with bacteria a slight decrease in viability when CO_2 was withheld for one or two days. The sensitive *Polytomella* dies without CO_2 after three to six days, but *Astasia* may be motile after 11 days.

Summary

Pure cultures of an alga (*Chlorella*) grown in the dark with organic food, and of two protozoa (*Polytomella* and *Astasia*) increased in number in hanging drop cultures in a normal atmosphere, but did not multiply when the carbon dioxide of the air was removed by alkali. The reason for the necessity of carbon dioxide for multiplication is unknown.

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FACTORS CONCERNED IN "GROWTH" AND "INFLAMMATION" OF METALS, GELS, PLANTS, AND ANIMALS[1]

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A. INTRODUCTION

This paper emphasizes similarity of reactions occurring in "growth" and "inflammation" of metals, gels, plants, and animals. The terms growth and inflammation are used in this comparison to emphasize similarity of reactions in living and non-living systems. Metals may be said to show growth as a result of inflammation due to corrosion with their resultant impairment or "diseased" condition.

Two phrases are used in our descriptions, reactionary zone and oxidation-reduction or *OR* mosaic. Reactionary zones are areas of activity showing, for example, increased metabolism, concentration of methylene blue, organismal growth, etc. The *OR* mosaic is the composition of the medium interpreted in apparent or potential Eh. This may vacillate as in the living cell with continuous shift from the more oxidized to the more reduced state. A disproportionate change, uncompensated, in one direction or in the other may result in death. If the change just unbalances the cell it may result in inflammation. Increase in alkalinity may decrease Eh, increasing the intensity of oxidizability while increase in acidity may increase Eh and preserve reducing substances to be released later on alkalinization with resultant irreversible or reversible oxidation. Ionization is an important factor (Harrow and Sherwin, 1935) in decrease in Eh on alkalinization (6a, 8a). It seems that in complex systems such as occur in living cells, other factors as spatial configuration, oxygen tension, carbon dioxide tension, variations of the larger entities, colloidal state, etc., play a considerable part. Demonstration of a gradient of Eh in alkalinized agar gel medium (Williams, J. W., 1939a, 1939b) suggests the possibility of a number of factors.

This work involves measurements of the reduced and oxidized states in terms of Eh under various conditions. It is correlated with

[1]Contribution No. 188, from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts.

present day knowledge and work performed previously. The following have helped initiate opinions and have emphasized its importance.

B. Corrosion of Metals

Corrosion results from a disturbing difference of potential of sites influencing each other. A reduced area in contact with oxygen under appropriate conditions will become corroded. A fresh metal surface on contact with the external atmosphere suffers corrosion of its surface in the form of a more oxidized film. This film may vary in nature either aiding further oxidation below or protecting the metal below by reducing permeability to oxygen. Metal plating is used to afford a film more impervious to oxygen. Impurities of metals and injuries allow formation of areas of difference of potential which in the presence of oxygen are ideal for corrosion. Table 1 is presented to illustrate difference of Eh on the surface and in the metal after the surface has been removed. The difference is apparent, the metal below being in a much more reduced state. In other words, a gradient exists from above down. There is no claim for accuracy of these readings. Scraps of metal were picked up, the purity of which was unknown. The only thing desired to illustrate is a trend. This trend can be compared with that of the skin of man (Williams, J. W., 1941a). In material more pervious to air, however, such as cured pine, whitewood, oak, no such marked difference is noted, the surface showing possibly a slightly greater Eh which might be considered due to variation in the instrument.

TABLE 1

Metal	Original surface		Surface removed	
	pH	Eh	pH	Eh
Aluminum	6.0	—.57	7.42	—.898
Copper	6.0	—.05	6.75	—.156
Iron	?	—.39	7.28	—.492
Steel	6.6	—.45	7.14	—.68
Brass	6.82	—.06	6.4	—.21
Bakelite	5.9	+.04	6.5	—.105

The pH and Eh were taken by applying the glass, calomel and platinum electrodes to the surface after cleaning it with ether and moistening the contact with Ringer's solution (pH 7.25, Eh + .05). While there was considerable vacillation, a point was reached which was comparatively stationary. The only purpose of this table is to show trend. The variation in Eh is considerable from time to time. The same applies to pH. The Beckmann potentiometer was used and the readings given are in volts using the calomel electrode as the reference electrode.

C. Changes in Gels

1. *Concentration of Dyes by Non-Living Substances* (5)

When methylene blue (0.003 to 0.001 per cent) is incorporated in a gel medium (agar 0.75 to 1.5 per cent) and autoclaved at 15 lbs. pressure for 20 min. a uniform blue color is developed throughout. When cysteine hydrochloride (0.1 to 1.00 per cent, pH 5.0 to 8.0) is incorporated in the medium, the medium at first colorless develops a line of dye concentration at the surface of the medium at the expense of loss of dye by the medium below. The width of the band of dye concentration (see Table 2) increases with decrease in concentration of cysteine, and with time not only becomes wider by added bands of dye but lines of more concentrated dye become apparent within the band. Tubes containing cysteine with a pH of less than 4.0 fail to solidify, are at first colorless, then pale green, then lose their color entirely. If sodium hydroxide is added to such tubes (exp. equal parts N/10 to 1.00 per cent cysteine) color is regained which disappears in several minutes. It seems that the initial sudden increase in alkali temporarily helps to oxidize methylene blue but liberates reducing potencies of cysteine, a marked lowering of Eh occurring shortly thereafter followed with slight increase, then lowering again. When distilled water was added in place of N/10 NaOH there was neither appearance of color nor significant change in Eh (see Table 3). The readings illustrate how alkali and (to a comparatively inappreciable degree) dilution increase the reduced state and coincidently apparently the avidity for oxygen, showing how both concentration and acidity tend to preserve substances of stored or dormant potential reducing power.

TABLE 2

Medium	24 hr. standing		8 days standing	
	Depth of dye	Other lines	Depth of dye	Other lines
No. 1 (1% cysteine)	1 mm.	——	2 mm.	——
No. 2 (.25% cysteine)	5.5 mm.	——	10 mm.	7
No. 3 (0.1% cysteine)	12 mm.	——	23 mm.	13-15, 5-6, 10-11, 20-21
No. 4	10 mm.	——		——
No. 5	12 mm.	——		——

No. 1—cysteine hydrochloride 1%, agar 0.75%, methylene blue 0.001%, pH 7.8
No. 2—cysteine hydrochloride 0.25%, agar 0.75%, methylene blue 0.001%, pH 5.5
No. 3—cysteine hydrochloride 0.1%, agar 0.75%, methylene blue 0.001%, pH 4.0
No. 4—d-glutamic 1%, agar 0.75%, methylene blue 0.001%, pH 8.2
No. 5—d-glutamic 1%, dextrose 1%, agar 0.75%, methylene blue 0.001%, pH 6.1
This table illustrated the depth of dye. In wide bands the concentration may show increase as lines within bands. With longer standing the bands become still wider and more dye lines appear within them.

TABLE 3

Medium	pH	Eh	Equal parts N/10 NaOH pH	Eh	Equal parts H_2O pH	Eh
Cysteine 1%						
Agar 0.75%	2.75	—.055	8.0	—.50		
M.B. 0.001%	2.4	—.074	9.5	—.45		
NaOH 0.2%						
Sample 3	1.8	+.02			2.1	+.01
Successive			7.7	—.65		
readings ¼				—.50		
to 2 min.				—.42		
				—.41		
				—.425		
4 minutes				—.54		
5 minutes				—.55		
15 minutes				—.58		
1 hour			8.2	—.50		
3 hours				—.57		
24 hours blue			8.6	—.082		

This table illustrates the variation which may occur in reduced state. This is not alone the case for mediums such as this but also for more complex mediums. In the process of inflammation there is a curve of Eh reaction which we are studying but which is too complicated to attempt an explanation at the present time. The medium above because of acidity is liquid. As will be noted there are several samples varying in acidity and Eh. It is only when NaOH is added that marked change occurs as demonstrated in readings of Sample 3.

More alkaline tubes (exp. pH 8.5) with time showed a band of green below the blue and a browning of the medium below this. Intense dye concentration often results in browning just below even to a pH of 7.0. (This seems to illustrate one importance of a reactionary zone in browning.) Very alkaline tubes (greater than pH 9.0) show a flocculate blue band, becoming a precipitate with further increase in alkalinity and finally becoming semi-solid and showing no green band below and very little browning. The browning of gels on addition of alkali will be discussed later.

The dye is apparently concentrated in the leuco form and changes to blue at the optimum potential and oxygen tension. This reactionary zone has characteristics which result in drawing up the dye independent of diffusion and the gradient of evaporation.

That other substances may act like less concentrated cysteine (0.1 per cent) with resultant wide dye line is shown when 1.00 per cent d-glutamic acid is substituted for cysteine (see Table 2).

2. *Concentration of Methylene Blue by Microorganisms*

(Williams, J. W., 1936, 1938a, 1938b, 1939c)

When certain aerobic bacteria are grown in a shake medium of nutrient agar containing 0.0003 per cent methylene blue they grow

near the surface gradually concentrating the methylene blue in or just below their growth. Successive bands of organismal growth and of dye may occur beneath the first if incubation is continued. The medium below remains colorless. Thus, like cysteine, bacteria produce a reactionary zone capable of dye concentration but in this instance growth of living organisms serves the purpose. By adjusting the ingredients it appears likely that the reactionary zones of cysteine and organisms can be made to correspond in appearance, site, and width. In addition, it is possible that the dye lines may be made to vary in color as a result of oxidation of methylene blue to various azures which is so apparent with certain organisms.

3. *Variation in Color of Gels*

As alkalinity (from pH 5.0 to 9.0) of agar (0.75 to 1.5 per cent, autoclaved) is increased brown color increases (see Figure 2). Likewise, when more alkaline mediums are placed under increasing oxygen tensions a deeper brown is present at the surface which increases in depth with increase in oxygen tension. In this more alkaline medium there is a gradient of Eh (Williams, J. W., 1939a, 1939b), the Eh decreasing up to a certain depth. When acid is added to such a medium or when it is exposed to ultraviolet light it tends to lose its color. This suggests a factor in addition to or other than caramelization as does the increase in depth of brownness with increase in oxygen tension. The possibility of change in the colloidal aggregates is being investigated. This in turn may be dependent on acidity.

4. *Liesegang Rings*

In the broad sense the dye bands and the bands of bacterial growth discussed previously can be considered examples of Liesegang Rings. Typical Liesegang Rings can be produced by including a dilute solution of one reactant in a gel and, on hardening, layering the gel with a concentrated solution of the other reactant. A periodic precipitation comparable in appearance to successive dye or growth bands occurs at successive depths on standing (Williams, 1939c). In all instances the successive bands may be due to removal of substances inhibiting, or provision of an essential for the next reaction; on analysis both assumptions may be correct. It is likely that Liesegang ring-like formations such as concentric rings in gallstones, stratification of some rocks, tree growth rings, weight gain in children in spring and summer, muscle contraction bands (Carey, E. J., 1940) may be dependent on completion of one reaction before the next is able to

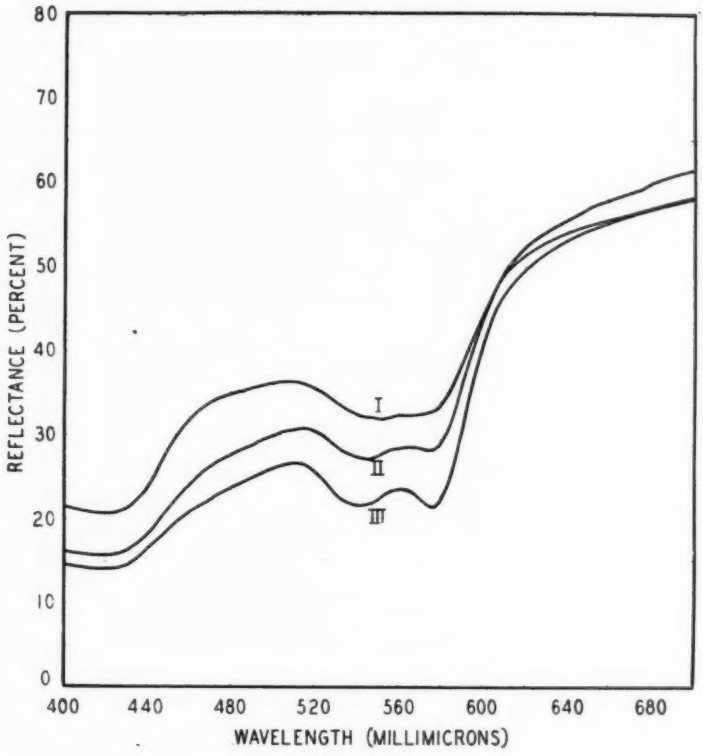

FIGURE 1

APPLICATION OF N/10 NaOH TO VOLAR SURFACE OF THE FOREARM OF MALE WHITE

1. Control.
2. Two layers gauze, saturated with NaOH solution, covered with adhesive, two days. Readings made.
3. Eight layers of gauze saturated with NaOH solution, covered with adhesive overnight. Readings made.

It must be emphasized that skins are not equally sensitive to NaOH. The larger number of layers of gauze resulted in a greater dosage of NaOH to the skin and one would expect a greater effect.

occur; such completion may be considered as removing inhibiting factors or contributing essentials.

D. CHANGES IN METHYLENE BLUE DEPENDENT ON SIMPLE SUBSTANCES (WILLIAMS, J. W., 1938c)

Relative amount of reduction of methylene blue (0.001 per cent)

contained in various aqueous solutions after being autoclaved at 15 lbs. for 20 minutes was studied by visual examination and by Hardy spectrophotometer readings. Solutions from pH 5.0 to 8.0 were compared. More acid and more concentrated solutions of cysteine (max. concentration 1.00 per cent) showed prolonged to indefinite reduction while dilution and alkalinity resulted in more immediate oxidation. In addition, alkalinity increased the active reduced state of many organic materials, converting them to a more oxidizable state (Williams, J. W., 1939b). More dilute cysteine solutions (0.25 per cent, pH 5.6) first showed fleeting oxidation, followed by reduction and later oxidation. At a concentration of cysteine between 0.125 per cent and 0.25 per cent a reversal of persistence of reduction occurred and more alkaline solutions (pH 8.0) showed more persistent reduction. This is a condition similar to that which exists when various amino acids or succinic, maleic, or fumaric acids are added to sugars such as dextrose, dextrin, sucrose, etc. With cysteine apparently a point of dilution is reached where reduction is sufficiently relatively decreased with reference to external conditions that comparable conditions result. Addition of d-glutamic acid to cysteine solutions (1.00 per cent) prolonged reduction only at the more acid reaction. When dextrose was added a condition of vacillating oxidation and reduction in acid solution resulted, the material reaching a state of neither comparatively complete oxidation or reduction. The latter state may be compared to that existing in the living cell.

The systems cited above may be termed "oxidation-reduction" or *OR* mosaics. It is evident that they can be built up into certain definite patterns having certain definite characteristics. Basic are certain types of carbon and nitrogen sources. To this must be added an appropriate atmosphere (Williams, J. W., 1938d) and the spark or growth essential whether it be within or extraneous to the organism or system. In this study, the catalysing influence of the reaction of amino acid and sugar was autoclaving and alkalinity and the indicator (possibly not without some function in the reaction) was methylene blue. This phenomenon was extremely simple, its complexity was increased by cysteine. Other substances could be added to make it more complex either by activating, inhibiting, or poising it in one fashion or another. Possibly functioning in the reaction were unidentified contaminants. Many *OR* mosaics of varying complexity can be visualized such as one in which oxidation is completed to a state of denaturation, one in which drying fixes the material in a

potentially oxidizable state as dormant seeds, one in which cells function and whose mechanism of oxidation-reduction is so balanced (Williams, J. W., 1939d) that there is no proliferation for the time being or one in which there is an excessive stimulus and the essentials of proliferation as in the growth of a malignancy.

The terminology *OR* pattern or mosaic implies a pattern characterized by energy potentialities which can be built up and modified according to circumstances and demands. Thus, we might modify the pattern of the system of a tetanus bacillus which would render it non-toxin producing or we might modify the pattern of a cell nucleus which either removes an inhibition to proliferation or supplies an essential. Since we know the importance of oxidation and reduction in these phenomena it seems that assumption of such patterns as dynamic and potential is logical.

In a previous publication (1939d), the author suggested a fifth dimension of space in addition to those of length, breadth, height, and time already accepted, i.e., equilibration. Without a balanced state things as they exist would be impossible. The *OR* mosaic can be considered a complex in a certain dynamic or potentially dynamic state under conditions defined. The tendency in the active mosaic is toward equilibration. A difference exists between it and its environments and it feels these effects in its avidity for hydrogen or oxygen or the equivalent. It is difference in state which maintains things in a balancing equilibrium.

E. Basic Factors in Growth of Microorganisms

Growth of microorganisms is dependent on the medium-atmosphere relationship and the microorganism (Williams, J. W., 1938d). Increase in oxygen tension increases depth of appropriate site for growth and vice versa. Carbon dioxide does likewise but also inhibits growth. Incubation at a colder temperature (limits) increases depth of appropriate site for growth possibly by allowing more ready penetration of oxygen or because the organisms initiating growth in unit time demand less oxygen either because there are fewer organisms establishing growth or the slowing of growth initiation demands less oxygen. Depth of growth in shake culture is also increased by a more oxidative medium, by presence of inhibiting substances resulting in growth of fewer organisms, by planting fewer organisms, by incubating the shake with growth of organisms on the surface, followed by incubation under increased oxygen tension, by the presence of oxygen dona-

tors or hydrogen acceptors in certain organismal systems, and possibly by increase in acidity of the medium. In many of the foregoing instances several sets of conditions act simultaneously. For example, increase in oxygen tension gives slight increase in acidity of the upper layers of the medium, while carbon dioxide produces considerable increase. Inhibitory substances and less organisms planted result in less demand for oxygen in unit area in unit time since less organisms establish growth; the organisms thus influence each other in the site of establishment of growth. This may help explain why a certain number of organisms are often essential for the establishment of growth. On reincubation at higher oxygen tension after growth has already taken place at the surface the deeper growth which occurs (as compared to a control in which no previous growth had occurred) is apparently due to conditions produced allowing more ready penetration of oxygen or removal of conditions inhibiting oxygen penetration. This explanation seems applicable to the successive bands of growth and dye concentration below the first on prolonged incubation or standing. It seems essentially to be the removal of inhibition to production of a reactionary zone below. Addition of essentials of growth as oxygen, nitrogen source, etc., may be necessary for growth or further growth. Conditions of hydrogen acceptance in certain organismal systems are shown by including methylene blue in the medium in which case the organisms grow as a band much deeper in the medium (Williams, J. W., 1939c); the reverse state of affairs is demonstrated when sodium lactate or sodium phosphate is added to a medium in which case organisms growing previously subsurface now grow on the surface (Williams, J. W., 1938e). One substance may be shown to act antagonistically to another. Thus, sulfanilamide increases the depth of the band of growth of certain organisms, while sodium lactate added to the medium antagonizes this effect. The above conditions illustrate modification of the *OR* mosaic and some of the essential conditions.

F. Plants

1. *Germination of Seeds*

Germination is encouraged by increased permeability to water, increased tension or permeability to oxygen, rupture of seed coat, sufficient development of the embryo, etc. The changes vary with the seed. Soil, temperature, altitude, etc., must be favorable for germination. Drying and concentration are essential in the formation of the

seed while in germination the reverse occurs. In drying potential oxidizable substances are preserved. On germination the material concentrated is released in energy by oxidation. An analysis indicates an *OR* mosaic in the seed dormant because of a state which lacks oxidizability. The seed only comes into its reactionary state on dilution with water, penetration of oxygen, or other conditions changing its constituents to a more oxidizable state.

2. *Eh and Plants*

Eh and pH readings were made on branches of lilac, rose, forsythia, and grape which were budding or about to bud. Ringer's solution was used to moisten the contacts. The bark was then scraped off. There was not a sufficient consistency of readings to indicate a trend. This may be due to the fact that there is only a thin surface zone of activity in these branches and that the potentiometer in measurement of the uninjured surface includes this zone. Since some readings appeared slightly higher after the branch was injured possibly some of the active zone was removed; in the absence of a compensatory reaction this appears probable. Further study is necessary in this field.

3. *Accumulations in Roots of Plants*

It has been established that salts (Hoagland. D. R., and T. C. Broyer, 1936) are attracted to plant roots in accordance with their metabolism. Prominent in these sequences are acidity and alkalinity. This attraction can occur independent of diffusion and seems dependent as in the previous cases cited, on a shifting but characteristic *OR* mosaic varying the intensity of the reactionary zone. Contact exchange in which ions jump from one colloidal particle to another is used in explanation of exchange in plant roots (Jenny and Overstreet, 1939; Broyer and Overstreet, 1940).

G. The Animal Body

1. *Localization of Inflammation*

Areas of inflammation are reactionary zones. If a dye such as trypan blue is injected intravenously it will localize in an area of inflammation (Burrows, 1932). Many other substances act likewise, many instances of bacterial localization in such sites being recorded. Mere rubbing or soaking in hot water may be sufficient for localization. Such localization is dependent on metabolic change and is

comparable to localization due to cysteine and bacteria. The *OR* mosaic is of such a nature that these occurrences become possible. There is a decrease of Eh and increased oxidizability. Circulation of blood to the inflammatory area increases, the body attempting to equalize excessive change in one direction or the other. In inflammation of the epidermis intercellular bridges become wider, the cells fuller, arranging themselves, especially the basal layer, more perpendicular to the surface so as to allow an easier exchange from the tissues to the outside and from the outside to the tissues below. Inflammation lowers the Eh (Williams, 1941b; Kelley; Downing; Williams) occasioning a greater avidity for oxygen which may be satisfied from the outside; experimental proof of increase of intake of oxygen from the outside has been difficult to obtain. Oxygen can reach areas of inflammation by the blood stream. It seems the *OR* mosaic in inflammation may well attract leucocytes as dyes were attracted in mediums studied. Changes concurrent with the reaction may well increase permeability (Williams, 1937). Inflamed cells or areas influence adjacent cells or areas until the condition is compensated. Just as there is a sequence of reactionary events in mediums so injured, cells by their changed avidities can attract oxygen in excess producing anoxia in adjacent cells with logical consequences. The Helmholtz double layer of the cell (and its colloids) will be disturbed with probable liberation of noxious or stimulating substances, and with disturbance of potential of the cell relative to its environs. Similar changes occur in non-cellular tissues. Proliferation results from elaboration of growth essentials or inactivation of inhibitors because of cell activity, cell death, or autolysis. Basically, the entire problem is concerned with shifting *OR* mosaics of varying intensity with resultant reaction, the body's intention being the reaccomplishment of the normal.

2. *Tanning of the Animal Body*

Inflammatory reactions such as due to sunburn or application of alkali produce a browning of the animal skin (Williams, 1941a). The reflectance spectrophotometric curve due to application of alkali to the human skin seems identical to that due to sunburn (Edwards and Duntley, 1939a) (see Figure 1). There seems evidence that tanning is due to oxidation. Since, however, the body is a gel and alkali produces not only its tanning but also browning of agar gel, other factors should be considered. Initial readings of agar gel medium

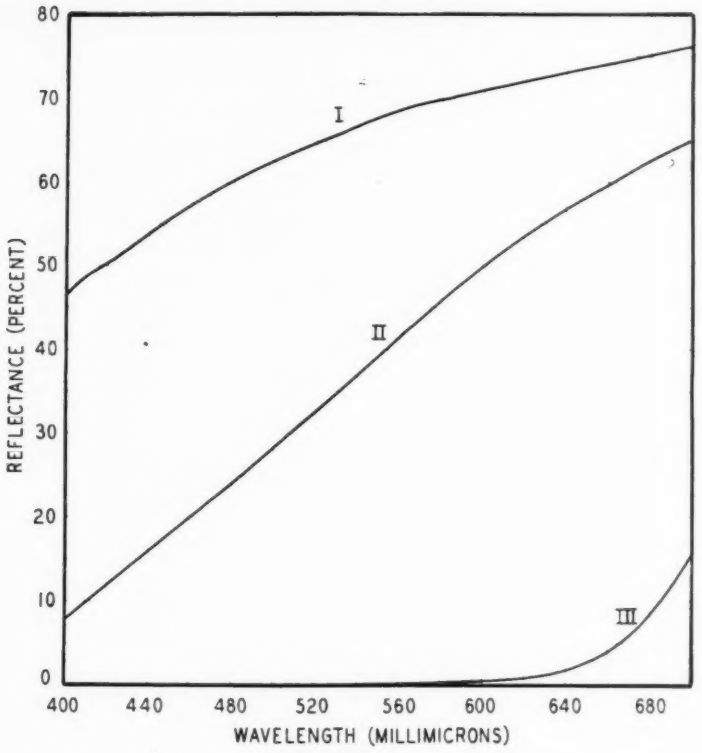

FIGURE 2

MEDIUMS AFTER BEING AUTOCLAVED WERE POURED IN 1 CM. INSIDE WIDTH SPECTROPHOTOMETRIC TYPE CONTAINERS AND ALLOWED TO SOLIDIFY AFTER WHICH THEY WERE READ

1. 0.75 per cent agar.
2. 0.75 per cent agar, 0.048 per cent NaOH.
3. 0.75 per cent agar, 0.48 per cent NaOH.

(Figure 2) show a more pronounced absorption in the violet. Like many pigments Beer's law may hold, i.e., the minus log of the transmission is in direct proportion to the concentration. The trend of the curves seems of an order similar to melanin which is so important in skin pigmentation (Edwards and Duntley, 1939b). Other inflammatory lesions also cause browning. There appears to be a quantitative relationship (increased) by lowered Eh and the persistence and amount of this condition together with the availability of oxygen,

between oxidizability of material, persistence of this oxidizability, intensity of the oxidation, and browning. The latter is dependent on the nature of the material present and any other materials which may gain access to the site and be influenced by or influential in the reaction. Acidity seems to be an antioxidant only insofar as it does not render material oxidizable or tends to stabilize it in a state which does not allow oxidation or as easy an oxidation (see Table 4). On the other hand, there is evidence acidity allows better permeability of oxygen.

H. Discussion

This paper has correlated phenomena in living and non-living mediums illustrating a significant similarity. Potentially more active

TABLE 4

Depth mm.	Medium 1		Medium 2	
	pH	Eh	pH	Eh
4	5.55	—.28	4.0	—.08
16	5.5	—.332	3.65	—.115
31	5.4	—.35	4.0	—.135
44	5.6	—.36	4.1	—.16

Both mediums contained 0.25 per cent cysteine hydrochloride 0.75 per cent agar, 0.001 per cent methylene blue and 0.64 per cent sodium hydroxide and were made as the same batch, the only difference being that they were in different test tubes.

The table is included to illustrate the small difference in pH and the comparatively large difference in Eh that may exist. As would be expected, the lower Eh and higher pH occur together.

materials whether they be living or non-living may show a gradient from without inward which can be measured as a decrease in Eh. The surface layer formed often serves as protective to a varying degree. In metals, in skin, and in gels such conditions are seen to exist. Disturbance in a normal gradient may result in inflammation and disease whether the entity is living or non-living. The ability of involved areas to influence adjacent areas should be especially emphasized. An inflamed area of the body because of its greater avidity for oxygen than an adjacent area may cause anoxemia and toxic reaction in such adjacent area if the body fails to compensate by increasing blood supply and satisfying the avidity.

Reactions are only reactions with reference to adjacent tissues or areas. They are reactions insofar as they call on their surroundings to satisfy their avidities. In such a manner differences are recordable. The cells which accumulate in the inflamed area can be considered attracted physicochemically by tissues showing an avidity for them

in satisfaction of the *OR* mosaic created. The satisfaction of such avidities may personify the reaccomplishment of the normal condition whether it be by hormones, vitamins, cells, or what not.

There is no method by which the zone of effect in measurement of Eh and pH of surfaces can be judged. The zone affecting the measurements may well be more extensive in certain metals and other substances than in others. This introduces a variable which is hard to control. Because of this fact one must judge one's readings in accordance and try for their interpretation with this in mind.

I. Summary and Conclusions

Similarities of reactions in living and non-living entities are stressed from a more basic point of view.

These reactions are in large part dependent on *OR* or oxidation-reduction mosaics in relation to their environs. These reactions occur in satisfaction of relative avidities present.

Acidity tends to preserve reducing substances which are recordable in a higher Eh (within limits). Alkalinization tends to release reducing potencies which are measurable in a lower Eh. Severity of the effects of the reaction of satisfaction seems dependent on reduction of Eh, persistence of this effect, substances affected, and intensity of reaction.

Reactionary zones to which particular substances are drawn other than by diffusion or gradient of evaporation are discussed. This attraction seems physicochemical dependent on the state of the material, its environs and the *OR* mosaics present. This data seems added reason why the physicochemical theory with reference to attraction of cells and other substances to sites of inflammation and the spread of inflammation should be further investigated.

The resemblance of reflectance spectrophotometric curves produced on application of sodium hydroxide to the skin to those produced by sunburn is discussed. The resemblance of transmission curves of melanin to those of alkaline agar gels also are discussed. Search for explanation is in progress.

Similarity of corrosion of the skin to that of metals is considered. Basic in both are differences in Eh.

The gradients of Eh in metals, gels, and the skin are discussed. The same trend of decrease from above downward to a certain depth is observable. No such dependable trend occurs with pH. The latter requires further study. No such definite Eh gradient is present

in cured wood or in budding twigs. The slight depth of the reactionary zone in twigs and the porous nature of the material is the probable explanation.

The building up of OR mosaics and their study using amino acids and sugars is discussed.

Theories presented to explain development of Liesegang rings and other rings such as bacterial growth "spectrums" are discussed.

For solution of problems considered, quantitation of active and potential Eh, pH effect, gel effect, environmental and adjacent effects and their relation one to the other are essential in the interpretation and understanding of *OR* mosaics.

The incomplete reversibility of the brown of alkaline agar gel medium produced by ultraviolet radiation or addition of acid may open up a new field of study of importance in colloidal chemistry.

The fact that a balancing state, that is, equilibration, is necessary for the existence of matter is re-emphasized. The suggestion that this be considered a fifth dimension of space is made.

It is impossible to include in this summary the contents of this paper. There are so many variables involved in the relationship of pH to Eh in complex systems that more detailed consideration is out of place here. This paper is concerned with correlations and with what can be gleaned thereby.

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